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Evaluating the protective effect of fluoride varnish on enamel subjected to pH cycling and toothbrush abrasion

Hathal Albagami

EVALUATING THE PROTECTIVE EFFECT OF FLUORIDE VARNISHES ON ENAMEL SUBJECTED TO DEMINERALIZATION AND TOOTH-BRUSH ABRASION

A Thesis Presented

By

HATHAL ALBAGAMI D.D.S.

Submitted to the College of Dental Medicine of Nova Southeastern

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MASTER OF SCIENCE IN DENTISTRY

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June 2018

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DATE SUBMITED: June 6, 2018

I certify that I am the sole author of this thesis, and that any assistance I received in its preparation has been fully acknowledged and disclosed in the thesis. I have cited any sources from which I used ideas, data, or words, and labeled as quotations any directly quoted phrases or passages, as well as providing proper documentation and citations. This thesis was prepared by me, specifically for the M.Sc. degree and for this assignment.

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Dedication

I would like to dedicate this thesis to my wife Dalal and out daughter Lana, who has supported me and stood by me the past three years. Her unyielding support and dedication to my comfort has been exemplary. I would also like to dedicate this thesis to my parents who were supportive every step of the way, I would not be here without them.

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Abstract

EVALUATING THE PROTECTIVE EFFECT OF FLUORIDE VARNISHES ON ENAMEL SUBJECTED TO DEMINERALIZATION AND TOOTH-BRUSH ABRASION

DEGREE DATE: JUNE 2018

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Objectives: To evaluate the protective effects of different fluoride varnishes on enamel subjected to pH cycling and toothbrush abrasion, via monitoring changes in enamel microhardness, and the measurement of wear after toothbrushing. **Methods:** A hundred and forty samples were prepared from thirty-five molar and seventy anterior teeth. Specimens were divided into six varnish groups (Fluor Protector S®, Vanish®, NUPRO® White, ProFluorid®, Duraphat®, and PreviDent®), and one Control group (*n*=20). Fluoride varnishes were applied according to manufacturers' instructions. Specimens were subjected to an 8-day pH cycling. The specimens were then exposed to 500 and 1,500 cycles of toothbrush abrasion to simulate one and three months of brushing respectively. Vickers Microhardness testing was done at baseline, after pH cycling, and after brushing for all groups. 3D digital scans were obtained from all specimens using an intra-oral scanner (CEREC OminCam®) at baseline, after 1 month of brushing, and after 3 months of brushing. Wear measurements were done using exocad® software. Surface examination was performed using Scanning Electron Microscope (SEM) to determine surface conditions at baseline, after pH cycling, and after toothbrush abrasion. Energy dispersion spectrometry (EDS) was used for the elemental analysis of the sample to quantify the presence of fluoride content at baseline, after pH cycling, and after toothbrush abrasion. **Results:** Fluor Protector S and Duraphat have displayed the highest surface microhardness values after pH cycling ($p<001$). Control group has shown significantly lower microhardness values compared to other groups ($p<001$). Fluor Protector S has shown significantly higher microhardness values after toothbrushing compared to other groups ($p<001$). Control group experienced significantly more enamel-wear than other groups ($p<001$), while Fluor Protector S and Duraphat groups showed the least amount of wear ($p<001$). **Significance:** Results obtained provide valuable information regarding the extent of the protection provided by fluoride varnishes for enamel. Unprotected enamel is prone to wear as a result of demineralization and toothbrushing, and the application of varnish appears to reduce the amount of wear, although results vary by varnish.

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Chapter 1: Introduction

1.1 Caries: Combating the disease with fluoride

1.1.1 Overview

Dental caries along with its consequences pose significant problems in industrialized societies and developing countries alike. Although the prevalence of dental caries has significantly decreased in the past two decades,[1] this disease is still common, and remains a great public health concern for a large number of the world's population.[2] Dental caries is a multi-factorial dynamic disease that involves the interaction between dental plaque containing bacteria, dietary habits, and host factors such as tooth surface characteristics, saliva, and the acquired pellicle.[3, 4] The bacteria in the plaque produces organic acids by metabolizing fermentable carbohydrates.[3] This results in the rapid decrease of pH from a physiological pH of 7.0 to a pH of 5.5, leading to an increase in hydrogen ion concentration more than 100-fold.[4] Hydrogen ions dissolve enamel minerals by diffusing into intercrystallite spaces through micropores in the enamel surface, leading to calcium and phosphate ions being free to move to the surface of the enamel, which eventually diffuse out of the tooth.[3] This is the demineralization process.

Remineralization is the natural repair response to demineralization. Remineralization is defined as "the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition ions crystal voids in demineralized enamel to produce net mineral gain".[5]

The tooth is continuously bathed in saliva under normal conditions. Saliva plays many important roles in enamel remineralization.[3] Phosphate, bicarbonate, and peptides in saliva buffer and neutralize acids produced by bacterial fermentation of carbohydrates causing the pH to rise back to neutral.[3] Furthermore, saliva can provide minerals that were lost during the demineralization process.[3] Saliva is usually supersaturated with calcium and phosphate ions and is capable of remineralizing the very early stages of lesion formation, specifically when fluoride is present.[3]

Since the introduction of water fluoridation, fluoride has been the key element of caries-preventive strategies. Research has shown that the caries preventive effect of fluoride is predominantly topical, which occurs as a result of fluoride uptake by the enamel. Enamel uptake is defined as the absorption of fluoride, its incorporation into the enamel, and its permanent or temporary retention. Caries-preventive function of fluoride occurs through promotion of remineralization, and reducing demineralization and loss of enamel minerals during early enamel lesions.[6]

1.1.2 Topical fluorides

Use of topical fluorides has increased over the past decades, with toothpastes, mouth rinses, gels and varnishes being the most widely used, either alone or in combination.[7] These topical applications have shown effectiveness in preventing and controlling dental caries.[8, 9] Topically applied fluoride is defined as “delivery systems which provide fluoride to exposed surfaces of the dentition, at elevated concentrations, for a local protective effect and are therefore not indented for ingestion".[10] Fluoride varnishes and gels are professionally applied topical fluorides, and are using in preventive programs.[10] Fluoride gels have also been used as a self-applied treatment in such programs.[10] The main forms of self-applied fluoride therapy are fluoride rinses and toothpastes.[10] Fluoride mouth rinses and their intensive use in school programs has been discontinued, due to doubts aimed at its' cost-effectiveness in low prevalence of dental caries.[10] They have been replaced by selective fluoride therapy, which is aimed at high risk children. Fluoride toothpaste is by far the most commonly used fluoride therapy,[9] and its' increased use has been linked to the decline in the prevalence of dental caries in developed countries.[1, 11]

Different topical fluoride delivery methods did not exhibit any significant difference when compared with single use or combinations.[7] However, several in-vivo and in-vitro studies have shown that varnishes supply fluoride more efficiently when compared to other modes of delivery.[12, 13]

1.1.3 Fluoride Varnish

Studies show that fluoride retention, reaction and release in the enamel are dependent on the duration of contact of the fluoride agent with the tooth structure.[14] Although no significant differences in caries preventive effect of different fluoride applications has been shown,[7] the ability of varnishes to adhere to tooth surfaces may make them the optimal option. In addition to their remineralization promotion through ion release, fluoride varnishes have been found to form a protective barrier on the enamel.[15]

Although there are various formulations of fluoride varnishes available, three formulations commercially available known as Vanish® (3M ESPE), NUPRO® White (Dentsply Sirona), and ProFluorid® (VOCO) currently lead the North American dental market.[16] These varnishes share the same active fluoride agent (5% NaF). While these materials share the same active ingredient with the same concentration of 2.26% weight fluoride (22,600 ppm), they differ in their delivery mechanism, which leads to different viscosities of these materials. Viscosity assures the right contact angle to achieve effective delivery of fluoride on the enamel surface.

A new material has been introduced commercially known as Fluor Protector S® (Ivoclar Vivadent). This material contains 1.5% ammonium fluoride in a varnish base with ethanol and water as solvents. The fluoride content is equivalent to 0.77% or 7,700 ppm. After application, the solvent evaporates leading to a fourfold increase in fluoride concentration (~30,000 ppm). It is claimed that this composition leads to the decrease of viscosity and improve wettability of the tooth surface, leading to improved enamel uptake. However, no independent study has been conducted to investigate the validity of this claim.

It has been suggested recently that a calcium fluoride-like material is deposited on enamel after the application of fluoride solutions and that it is responsible for the cariostatic action of topical fluorides.[17] This morphological change in the surface of enamel theoretically leads to the improvement of the physical properties, mainly resistance to deformation and maintaining morphological integrity of the surface of enamel, which potentially leads to improved resistance to enamel surface loss and deformation.

1.2 Enamel Surface Microhardness (SMH)

Enamel surface hardness is defined as the resistance of the surface to scratches, abrasion, and indentation, as well as the resistance to elastic and plastic deformation at the time of force exertion.[18] Microhardness testing is widely used to evaluate the hardness of different materials, and is commonly used to evaluate the physical properties of tooth structures.[19-21] Vickers hardness testing and Knoop hardness testing are the most widely used in dentistry among the different methods available.[22] Microhardness testing is valuable to evaluate teeth undergoing demineralization, as the loss of minerals results in decreased surface microhardness and enamel.

1.3 Tooth Brushing and Abrasion

The role of plaque accumulation on tooth surfaces in the promotion of caries and periodontal disease is well-established. The most common, accessible, economic, and self-performed method of meticulous plaque removal is tooth-brushing.[23] Since its introduction in 1857 by H. N. Wadsworth, evidence confirm that toothbrushing executed at regular and appropriate intervals is an effective tool in controlling dental plaque. Thus, the use of a mechanical toothbrush with fluoridated toothpaste is one of the best means of self-employed plaque control. However, if toothbrushing is not done properly, it has the potential to cause abrasion of soft tissues, as well as hard tissues of the oral cavity.[24]

Dental abrasion was first described by Zsigmondy in 1894 as angular defects, and was later described in 1907 by Miller as wasting of tooth structure. Abrasion is recently described as non-carious cervical lesions, along with erosion and abfraction. [25] Dental abrasion is defined as the mechanical removal of hard tooth structure by repeated introduction of foreign bodies that are in contact with the tooth surface.[26] The epidemiology of tooth abrasion is not very well known, and most of the evidence comes from the examination of skeletal remains.[27] There are no investigations linking the prevalence of dental abrasion to a sample which is representative of a population group.[28] The prevalence of cervical lesions has been shown to increase with age, with toothbrushing being the most likely cause.[29] While dental abrasion could happen on any tooth surface, it most commonly appears on the buccal cervical region of incisors, canines, and premolars of both jaws.[30]

Toothbrushing is often associated with abrasion of teeth in the cervical area, however, the effects of toothbrush abrasion on sound enamel is negligible.[30, 31] However, abrasion of enamel softened by the effect of acid erosion could happen with just a few strokes of the toothbrush, due to its fragile state.[32] Toothbrush abrasion and erosion have been shown to be additive and synergistic in their effect in different *in situ* studies.[32, 33]

Toothbrushing with a tooth paste can cause damage to oral hard tissues depending on their degree of abrasivity, specifically those with relative enamel abrasivity (REA) values above the recommended levels. In regards to the abrasivity of whitening toothpastes vs conventional, results vary. Some studies conclude that the whitening toothpastes do not cause more wear when compared to conventional ones.[34, 35] Another study has shown that whitening toothpastes cause significantly more wear than conventional variants.[36]

Dentine has been shown to be significantly more susceptible to abrasion than enamel.[33] However, analysis of in vitro data shows that to remove 1 mm of sound enamel, it would take hundreds of years.[27] Overzealous toothbrushing maybe responsible for a small percentage of cases of tooth wear and dentin hypersensitivity.[33]

1.4 Dental wear quantification using intra-oral scanning

1.4.1 Overview

The increase in life expectancy has made dental wear increasingly relevant as teeth are retained in the mouth longer. The main drawback of dental wear studies is that they are often based on subjective evaluation, such as scoring systems, questionnaires, and self-reports.[37-39] The most commonly used methods for wear evaluation are wear indices such as Smith and Knight,[40] which are incapable of determining wear progression.[38] The number of studies conducted to quantify tooth wear has increased in recent years.[41-43] Several studies investigated tooth wear using optical images, in which they used methods that required the acquisition of the images from physical models.[41, 44, 45] The most prevalent methods of surface analysis currently are optical profilometry and laser scanning.[46-48]

In recent years, to avoid inaccuracies resulting from working with physical models, intra-oral imaging techniques and devices have been developed for computer-assisted manufacturing of dental restorations. Recently, intra-oral digital scanners have been used to quantify surface loss after wear.[49, 50] 3D models of the test surfaces are generated, and analysis of the surface changes calculated using a specialized software. Practicality and availability of intra-oral scanners compared to expensive and narrow application of other wear measurement devices makes them a logical alternative. This innovative approach allows repurposing of the scanners for the use on different software for surface analysis of *in vitro* and *in vivo* applications.

1.4.2 CEREC OmniCam®

Sirona's CAD/CAM technology has been leading the market the longest when compared to the other digital scanning systems, and have existed for the past 30 years. Their latest system is the CEREC AC with OmniCam (Sirona, Bensheim, Germany), which was released in the summer of 2012. The OmniCam creates images using powderless scanning, creating a full-color model, as opposed to its' predecessors, which generated a yellow stone-like model via stitching together individual images. The accuracy and clinical acceptability of OmniCam is well documented.[51, 52] Utilization of OmniCam *in vitro* is beneficial for accurate, reproducible, and time saving scans. A recent study found that the software version of the OmniCam has a significant effect on the accuracy of scans, showing that version 4.4.4 has the least deviation.[53] They conclude that it is imperative to publish the version of the software used when conducting a study using OmniCam.

Surface changes of enamel happens on the micron level, and usually displays an irregular pattern. Therefore, a precise method is needed to obtain measurements with accuracy. Powder-free scans are beneficial in this type of study, to prevent surface alteration for accurate measurement of wear on the micron level.

1.4.3 Using exocad® software for analysis of scans

exocad® (exocad GmbH, Darmstadt, Germany) uses a combination of manual reference point selection, as well as a best fit match algorithm for the superimposition of the different scans. The software displays surface changes between the different scans using a color scheme ranging from blue (no difference), up to purple which indicates the most change. Tolerance of surface change detection can be adjusted to as low as 1μm. Quantification of surface wear is conducted using a measurement tool (Figure 1).

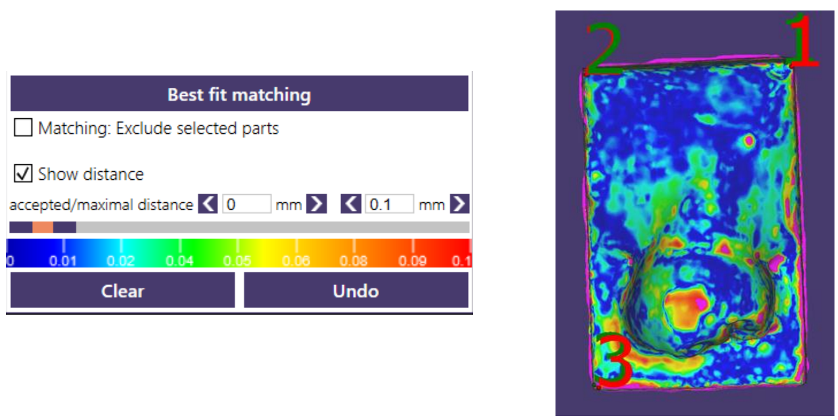


Figure 1 showing the color scheme displayed after superimposition of the scans

1.5 Innovation

Fluoride varnishes have proven to be the most effective and efficient form of topical fluoride delivery in terms of remineralization promotion, and resistance to acidic challenge.[7, 54, 55] Their use is widely accepted and employed in everyday dental practices. They were originally developed to increase the contact time between the fluoride agent and tooth structure by way of their adhesion to the surface, and new chemical composition of fluoride varnishes continue to be developed to improve upon that original concept. While many researchers have investigated fluoride in all of its aspects, a review in the chronological trends show that the most researched compounds are NaF and SnF_2.[56]

Taking this information into account, we believe that this study is innovative as it aims to evaluate the resistance of varnish applied on enamel to physical abrasion caused by daily toothbrushing, which, to the author's best judgement, has not been investigated yet. Our study employs new innovative techniques for the measurement of enamel wear by utilizing 3D scans obtained via an intraoral scanner, and performing the quantification using a state-of-the-art software.

This study includes less researched fluoride compositions such as ammonium fluoride. Decreased viscosity and the main fluoride component of this varnish is worth investigating in terms of improvement in fluoride uptake and film retention of the varnish on the enamel surface, which consequently lead to tangible improvement in the physical properties of the enamel.

Results obtained from this study will affect the dental professionals' decision when selecting the proper fluoride varnish. Comprehensive understanding of different fluoride varnish compositions, and viscosities are important factors to be considered when applying these materials.

1.6 Purpose of the study

The purpose of this study is to evaluate the effectiveness of different topical fluoride varnishes on enamel that is challenged with demineralization/remineralization cycling, as well as abrasion from toothbrushing. The study also aims to employ a new digital scanning method to quantify surface wear of the enamel using a specialized software for accurate measurement.

1.7 Specific aims and hypotheses

1.7.1 Specific aims:

I. To evaluate the change in surface microhardness of enamel treated with fluoride varnishes, and then subjected to pH cycling and toothbrush abrasion.

II. To quantify the surface loss of demineralized enamel after being subjected to toothbrush abrasion.

III. To compare the effectiveness of different compositions of fluoride varnishes to protect and preserve the integrity of the enamel surface after pH cycling and toothbrush abrasion.

1.7.2 Hypotheses:

I. Ho: Enamel treated with fluoride varnishes will show improved resistance to demineralization and toothbrush abrasion when compared with the control group.

II. Ho: Ammonium fluoride based varnish will show improved protection of the enamel in terms of demineralization and wear resistance.

1.8 Location of the study:

The design, preparation, data collection, and analyses for this study took place at:

Bioscience Research Center, Room 7356

Nova Southeastern University

Health Professional Division

College of Dental Medicine

3200 South University Drive

Fort Lauderdale, Florida 33328-201

Chapter 2: Materials and Methods

2.1 Sample size

The sample size was determined based on similar articles that were done in relation to the effect of fluoride varnish uptake, microhardness, and remineralization potential

- Vicente et al, 2017[57] to evaluate quantitatively and qualitatively the changes produced to enamel after interproximal reduction and subjected to demineralization cycles, after applying a fluoride varnish. It was decided that the number for each study group will be n= 23 per fluoride varnish used.
- Majithia et al, 2016[58] to compare and evaluate the remineralization potential of three commercially available varnishes on artificial enamel lesions. Vickers Microhardness test was done to evaluate the change in physical properties of the enamel surface. It was decided that the number for each study group will be n= 20 per fluoride varnish used, and one group served as control.

A total of 140 specimens were prepared. They were randomly divided into 7 groups of 20 specimens each per group. This includes an additional 2 specimens per group added to take into account error in specimen fabrication.

2.2 Sample preparation

The specimens were handled following the IRB regulations of Nova Southeastern University (IRB #: 2018-197). Sound human teeth were collected from an unidentified bank of teeth located in the Biomaterials Research Laboratory. Thirty five molars and seventy anterior caries-free teeth were selected for this study. Selected specimens were inspected for caries and enamel cracks under optical microscope (SZX7, Olympus, Tokyo, Japan), and specimens that showed evidence of caries and cracks were excluded (Figure 2). Molars were sectioned into buccal and lingual halves using Buehler sample preparation machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) (Figure 3). Teflon mold corresponding to the holders of the toothbrushing machine was used for the fabrication of acrylic blocks (Figure 4). Red acrylic resin (Ortho Jet, Lang Dental Manufacturing Co., Wheeling, IL, USA) was poured and allowed to set for 1 hour. After taking the blocks out of the mold, the surface was gently flattened using Buehler sample preparation machine (MetaServ 2000, Buehler, Lake Bluff, IL, USA) down to 1,200 grit size sandpaper (Figure 5).

Specimens were mounted on top of the acrylic blocks using impression compound (Type I, Kerr Corporation, Orange, CA, USA). A 2-step nail polish (Gel Couture, Essie Cosmetics Ltd., Astoria, NY, USA) was applied to the specimen covering the roots, as well as the contact area between the specimen and the acrylic block, leaving the crown of the tooth exposed (Figure 6). A small and superficial flat area on the enamel surface was created by using Buehler sample preparation machine (MetaServ 2000, Buehler, Lake Bluff, IL, USA) down to 1,200 grit size sandpaper, to allow for accurate microhardness measurements.

2.3 Study groups

Specimens were randomly divided into 7 groups (n=20), with each group comprised of 10 anterior teeth and 10 molars. Fluoride varnishes (Table 1) were applied to the surface of the enamel according to manufacturers' instructions for six groups, and one group served as negative control.

Specimens were placed in artificial saliva (0.1029 g $CaCl_2$ $2H_2O$, 0.04066 g $MgCl_2$, 0.544 g KH_2PO_4, 4.766 g Hepes buffer acid form, 2.2365 g KCl in 1000 ml distilled water/ pH 7),[59, 60] and stored in an incubator for 24 hours at 100% humidity.

After removal of the samples from the artificial saliva, the fluoride varnish was carefully removed using a toothbrush, standardizing varnish loss for all groups.

Product Name	Composition	Lot Number	Expiration Date	Manufacturer
Fluor Protector S	NH_4F	W02278	25-12-2019	Ivoclar Vivadent
Vanish	NaF	N910440	28-7-2019	3M ESPE
NUPRO White	NaF	1705211	28-5-2019	Dentsply Sirona
ProFluorid	NaF	1636500	28-9-2-18	VOCO
Duraphat	NaF	189879	1-1-2020	Colgate Palmolive
PreviDent	NaF	63060BD3HM	31-10-2018	Colgate Palmolive

Table 1 showing the different fluoride varnishes used for six of the study groups.

2.4 Demineralizing/remineralizing solution preparation

Buffered remineralizing and the demineralizing solutions were synthesized using analytical-grade chemicals and distilled water. The demineralizing solution, which contains 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, 0.05M acetic acid had the pH adjusted to 4.4 with 1M NaOH. The remineralizing solution, which contains 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M had the pH adjusted to 7.0.[61] This solution approximates to the super saturation of apatitic minerals found in saliva.

2.5 pH cycling model

During each day of the total 8-day cycle, the specimens were immersed at 37°C in an incubator with an orbital shaker (1575 Incubator Shaker, VWR, Miami Lakes, FL, USA) in the demineralizing solution for 2 hours, and for 22 hours in the remineralization solution, with the shaker set to 60 rpm (Figure 7).[62, 63] The demineralization and remineralization solutions were replaced bidaily throughout the cycle.

2.6 Toothbrush abrasion protocol

Samples were exposed to 500 and 1,500 cycles of toothbrush abrasion to simulate 1 month and 3 months of brushing respectively,[64] using an automatic brushing machine (V-8 Cross Brushing Machine, Sabri Dental Enterprises Inc., Downers Grove, IL, USA) (Figure 8). Brushing heads were fitted with nylon bristles (Oral B, Procter &Gamble, Cincinnati, OH, USA). Care was taken to ensure that bristles were perpendicular to the surface of each sample and touch the surface evenly. A 50:50 (w/w) slurry of 56.6g of non-fluoride toothpaste (Tom's of Maine, Fluoride-Free Natural Antiplaque and Whitening

Toothpaste) and 56.6ml deionized water was used as abrasive medium. Each surface was brushed using a brush head contact force of 300 grams. The slurry was refreshed for each sample for standardization.

2.7 Surface microhardness testing (SMH)

SMH of the specimens was determined using Vickers microhardness testing machine (Mitutoyo, Kawasaki, Japan) at baseline, after pH cycling, and after toothbrush abrasion. A load of 100 g was exercised steadily to the flat surface of the specimens for 10 seconds using Vickers diamond pyramid indenter under a x40 objective lens (Figure 9).[65] Three measurements were taken for each sample at each interval.

2.8 Surface wear measurement

Specimens were scanned using OmniCam® (Sirona, Bensheim, Germany) to obtain 3D digital models of the specimens at baseline, after 1 month brushing, and after 3 months of brushing. The 1 month and 3 month scans of each specimen were compared to its corresponding baseline scan. Superimposition of the scans and wear quantification was conducted using exocad® software (exocad GmbH, Darmstadt, Germany) via a best-fit match algorithm (Figure 10). The area of maximum loss was identified on the surface of the specimen, and 3 measurements were taken. The mean maximum loss was calculated for each specimen at 1 month and 3 months compared to the baseline.

Accuracy of the software measurement was tested by repeating the superimposition process 10 times, and measuring the same exact point after each time. The test yielded an accuracy rate of ± 6 µm, which is approximately a 5% error.

2.9 Scanning electron microscope (SEM) and energy dispersion spectrometry (EDS)

The most commonly used methods for evaluating anti-caries potential of fluoride agents in vitro is the simulation of physicochemical effect of fluoridated products.[66, 67] Said effect is best evaluated by observing the enamel specimens under the SEM to detect and analyze morphological surface changes, and using EDS to evaluate the specimens for changes in the chemical composition of the enamel.

One baseline sample, one pH cycling sample, and one after three-months brushing sample from each group selected for morphological analysis using SEM, and elemental analysis using EDS (XL 30 ESEM-FEG, Philips, Amsterdam, Netherlands) (Figure 16). Specimens were coated with Palladium prior to analysis (Sputter Coater 108 Auto, Cressington Scientific Instruments, Waltford, England) (Figure 17). Three SEM images were obtained for each sample at 5000, 10000, and 20000 magnification. Qualitative and quantitative analyses were conducted to evaluate the minerals present on the surface of the enamel, and to quantify the presence of fluoride at baseline, after pH cycling, and after brushing.

2.10 Statistical analysis

2.10.1 Microhardness

Means and standard deviations are calculated for all continuous measures. To compare differences for the outcome measure microhardness, a two-way ANOVA model with interaction was created. The fixed effects were varnish (Control, Duraphat, Fluor Protector S, NUPRO White, Prevident, ProFluorid, Vanish), time (baseline, after brushing, after pH cycling) and the interaction of varnish by time. Post-hoc tests were conducted using a Holm adjustment. RStudio and R 3.2.4 were used for all statistical analysis, and significance was accepted at $p<0.05$.

2.10.2 Enamel wear

Means and standard deviations are calculated for all continuous measures. To compare differences for the enamel-wear outcome measure, a two-way ANOVA model with interaction was created. The fixed effects were varnish (Control, Duraphat, Fluor Protector S, NUPRO White, Prevident, ProFluorid, Vanish), time (1-month, 3-months) and the interaction of varnish by time. Post-hoc tests were conducted using a Holm adjustment. RStudio and R 3.2.4 were used for all statistical analysis, and significance was accepted at $p<0.05$.

Figure 2: Optical microscope (SZX7, Olympus, Tokyo, Japan) used for inspection of samples for caries and enamel cracks.

Figure 3: Buehler sample preparation machine (IsoMet 1000) used for sectioning of molar samples into buccal and lingual segments.

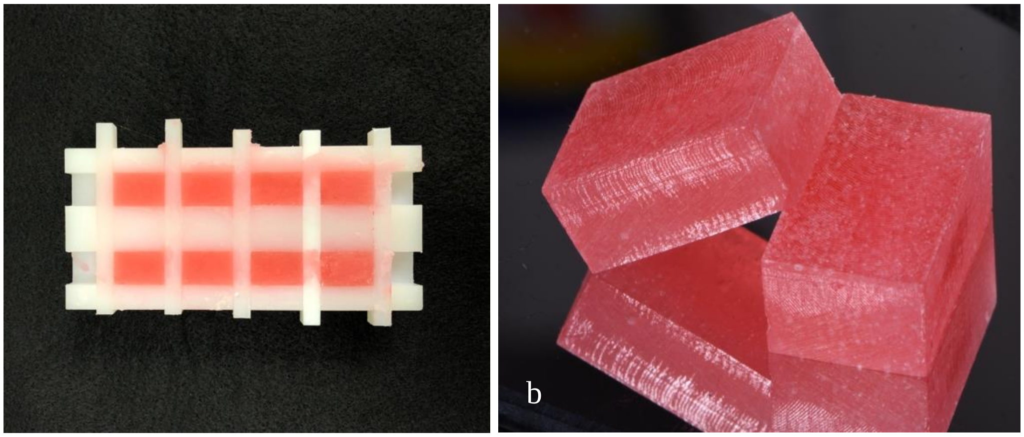


Figure 4: a) Teflon mold using for the fabrication of the acrylic blocks. b) Finished and polished acrylic blocks.

Figure 5: Buehler sample preparation machine (MetaServ 2000) used for finishing and polishing of samples.

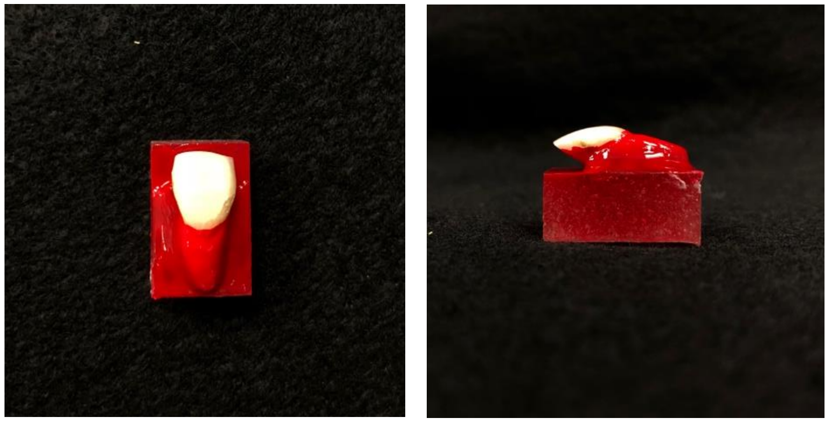


Figure 6: Two different views of the assembled samples.

Figure 7: Incubator (1575 Incubator Shaker) used for pH cycling of the samples.

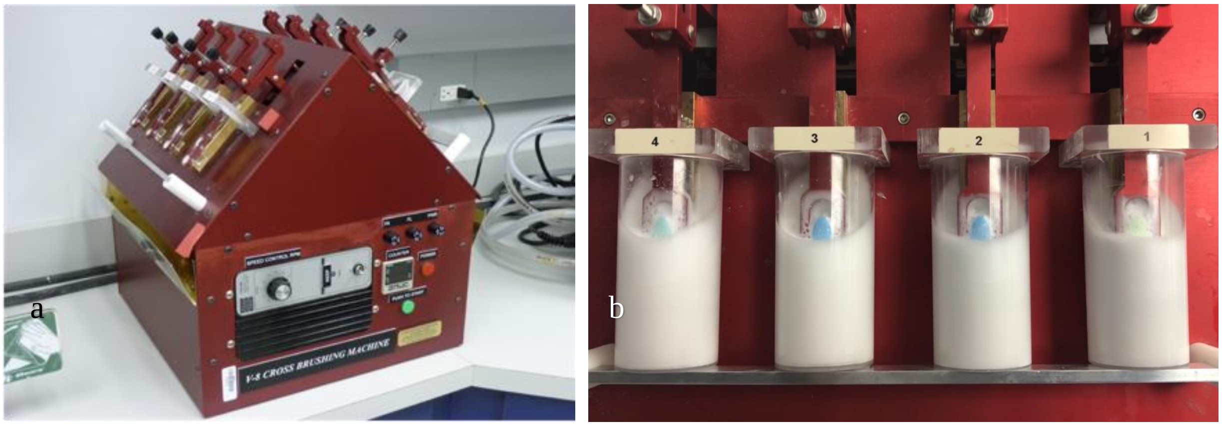


Figure 8: a) Automatic brushing machine used for toothbrushing simulation. b) The specimens mounted under the toothbrushes and submerged in toothpaste/water slurry.

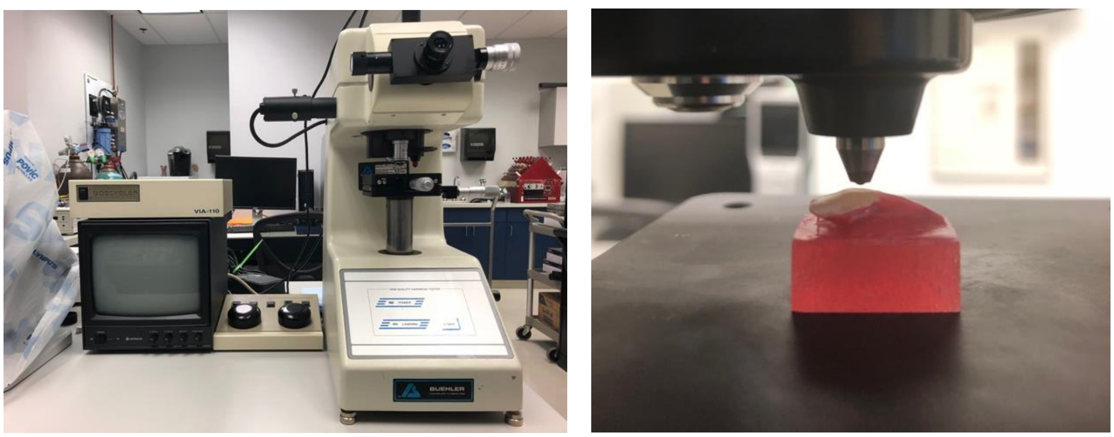


Figure 9: a) Vickers Microhardness testing machine. b) Sample placement at the time of force excretion.

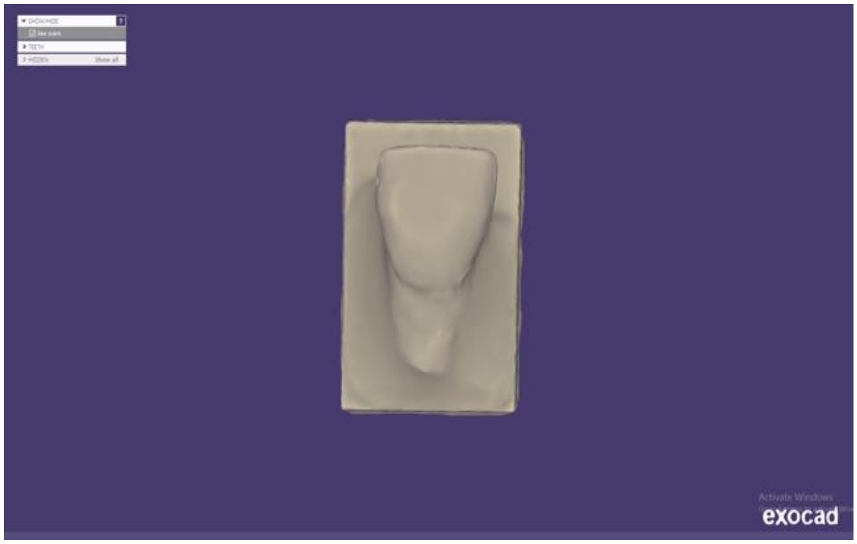


Figure 10: Wear measurement process using exocad software. STL files of 3D scans imported to exocad

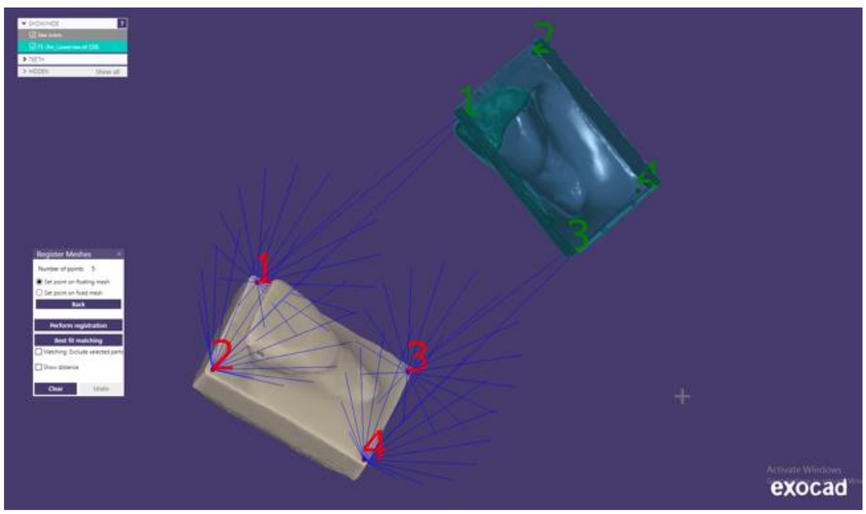


Figure 11: Wear measurement process using exocad software. Initial superimposition of scans using manual method.

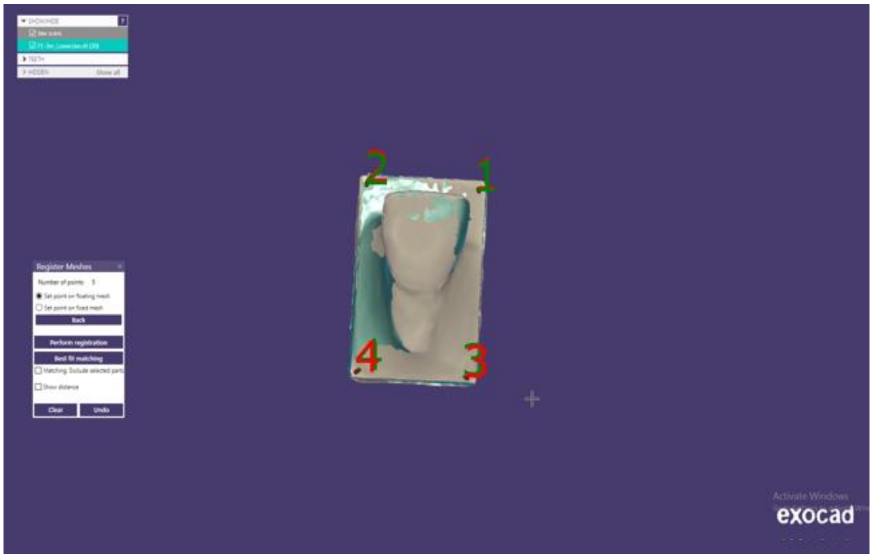


Figure 12: Wear measurement process using exocad software. Initial superimposition.

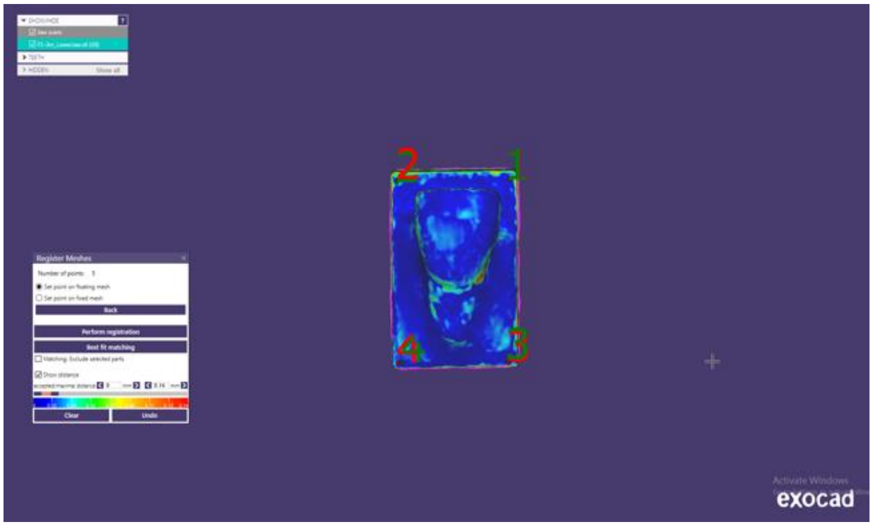


Figure 13: Wear measurement process using exocad software. Best fit match used for final superimposition.

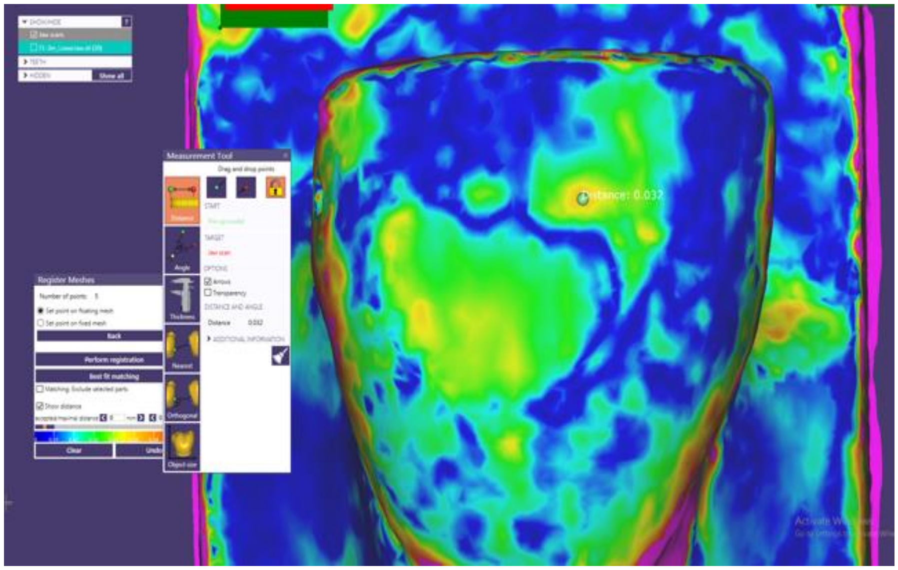


Figure 14: Wear measurement process using exocad software. Selection of areas with highest amount of enamel-wear.

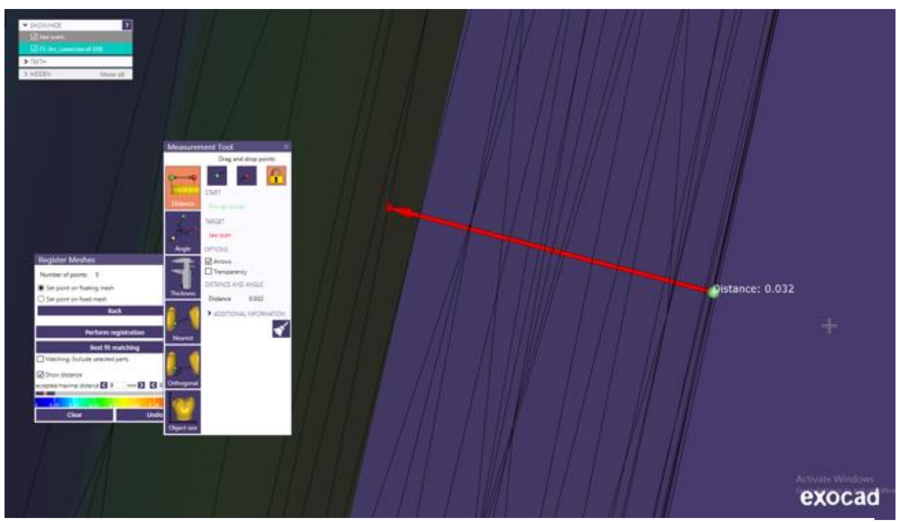


Figure 15: Wear measurement process using exocad software. Final measurement of vertical enamel-wear.

Figure 16: SEM and EDS used for surface and chemical analysis of the samples.

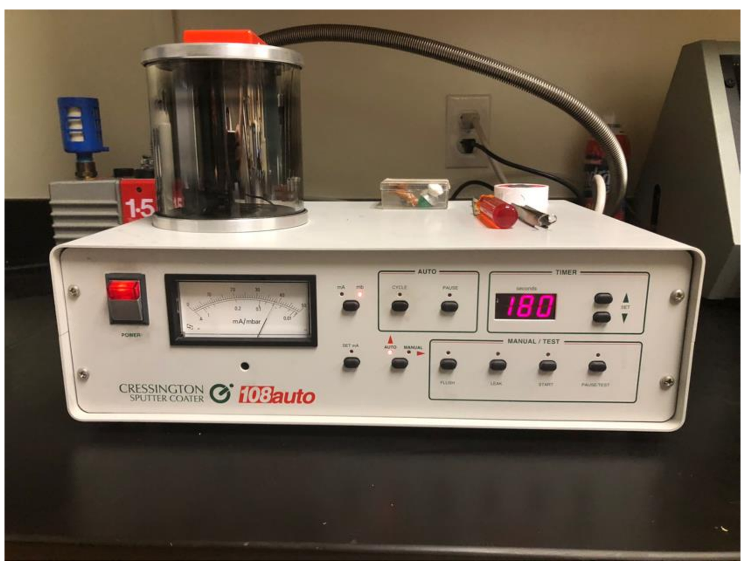


Figure 17: Sputter coater used to coat the samples with Palladium.

Chapter 3: Results

3.1 Microhardness

There was a significant difference in the measurement of *microhardness* by varnish $F[6,392) = 28.35$, $p<0.001$, eta-squared = 2.5% - meaning 2.5% of the variability in *microhardness* was accounted for by the differences in varnish]. There was a significant difference in the measurement of *microhardness* by time $F[3,392) = 2990.84$, $p<0.001$, eta-squared = 89.9% - meaning 89.9% of the variability in *microhardness* was accounted for by the differences in time]. There was a significant difference in the measurement of *microhardness* by varnish and time (Figure 11), $F[12,392) = 9.00$, $p<0.001$, eta-squared = 1.6% - meaning 1.6% of the variability in *microhardness* was accounted for by the differences in varnish over time]

After pH cycling, Fluor Protector S group has shown significantly higher SMH than NUPRO White ($p<0.001$), ProFluorid ($p<0.001$), Vanish ($p<0.001$), and Control groups ($p<0.001$). Although no significant difference was observed between Fluor Protector S, Duraphat, and PreviDent, Fluor Protector S has displayed the highest average SMH. Between all varnish groups, Vanish has shown significantly lower SMH than all groups. Control group has shown significantly lower SMH when compared with all varnish groups ($p<0.001$) except for Vanish group ($p=0.269$).

After brushing abrasion, Fluor Protector S displayed significantly higher SMH when compared to all other groups ($p<0.001$). No significant differences were detected between the other groups.

Detailed descriptive statistics are provided on Table 2. Pairwise comparisons are provided in Table 3.

3.2 Enamel wear

There was a significant difference in the measurement of *enamel-wear* by varnish $F[6,252) = 38.59$, $p<0.001$, eta-squared = 43.5% - meaning 43.5% of the variability in *enamel-wear* was accounted for by the differences in varnish]. There was a significant difference in the measurement of *enamel-wear* by time $F[1,252) = 44.83$, $p<0.001$, eta-squared = 8.4% - meaning 8.4% of the variability in *enamel-wear* was accounted for by the differences in time]. There was no significant difference in the measurement of *enamel-wear* by varnish and time (Figure 12) $F[6,252)=0.58$, $p=0.763$, eta-squared = 0.6% - meaning 0.6% of the variability in *enamel-wear* was accounted for by the differences in varnish over time].

After 1 month of brushing abrasion, Control group has shown significantly more enamel wear than other varnish groups ($p<0.001$), except for ProFluorid ($p=0.341$). Fluor Protector S has displayed the most resistance to brushing abrasion, showing significantly less enamel wear than Prevident ($p=0.037$), Vanish ($p=0.046$), ProFluorid ($p<0.001$), and Control ($p<0.001$) groups. However, no significant differences were detected between Fluor Protector S and Duraphat ($p=1.0$) or NUPRO White ($p=0.565$).

After 3 months of brushing abrasion, Control group has shown significantly more enamel wear than other varnish groups ($p<0.001$). Fluor Protector S has displayed the most resistance to brushing abrasion, showing significantly less enamel wear than Prevident ($p=0.007$), Vanish ($p=0.009$), ProFluorid ($p<0.001$), and Control ($p<0.001$) groups. However, no significant differences were detected between Fluor Protector S and Duraphat ($p=1.0$) or NUPRO White ($p=0.386$).

Detailed descriptive statistics are provided on Table 4. Pairwise comparisons are provided in Table 5.

Table 2. Descriptive statistics for microhardness

Varnish	Time	N	Mean	SD	Min	Max
Control	Baseline	20	303.15	27.79	226.97	358.17
Control	After pH cycling	20	12.31	4.84	5.90	27.97
Control	After Brushing	19	269.81	32.36	191.23	308.50
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
Duraphat	Baseline	20	308.64	45.31	232.43	408.10
Duraphat	After pH cycling	20	97.24	10.18	80.63	121.17
Duraphat	After Brushing	19	287.36	31.35	239.47	352.63
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
Fluor Protector S	Baseline	20	328.21	42.91	217.03	438.20
Fluor Protector S	After pH cycling	20	104.26	9.15	89.30	123.30
Fluor Protector S	After Brushing	19	331.16	34.11	275.73	397.47
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
NUPRO White	Baseline	20	329.72	39.25	254.13	381.77
NUPRO White	After pH cycling	20	55.92	8.13	46.07	69.47
NUPRO White	After Brushing	19	266.49	47.77	162.90	332.50
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
Prevident	Baseline	20	377.59	48.71	273.00	423.90
Prevident	After pH cycling	20	77.29	10.31	63.57	102.27
Prevident	After Brushing	19	298.52	48.79	224.27	395.83
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
ProFluorid	Baseline	20	313.02	30.53	260.70	355.27
ProFluorid	After pH cycling	20	48.71	11.74	23.80	69.13
ProFluorid	After Brushing	19	276.88	35.92	221.17	332.87
Varnish	**Time**	**N**	**Mean**	**SD**	**Min**	**Max**
Vanish	Baseline	20	339.05	26.33	283.70	378.03
Vanish	After pH cycling	20	34.41	5.33	22.27	44.73
Vanish	After Brushing	19	276.21	20.80	237.93	309.60

Table 3. Pairwise comparisons for microhardness

	Baseline Mean Difference	Baseline P.Value	After pH Cycling Mean Difference	After pH Cycling P.Value	After Brushing Mean Difference	After Brushing P.Value
Control - Duraphat	-5.48	0.998	-84.93	0.000	-17.55	0.586
Control - Fluor Protector S	-25.06	0.143	-91.96	0.000	-61.35	0.000
Control - NUPRO White	-26.57	0.098	-43.61	0.000	3.32	1.000
Control - Prevident	-74.44	0.000	-64.98	0.000	-28.71	0.068
Control - ProFluorid	-9.86	0.952	-36.40	0.004	-7.06	0.992
Control - Vanish	-35.90	0.005	-22.10	0.269	-6.39	0.996
Duraphat - Fluor Protector S	-19.58	0.418	-7.02	0.992	-43.80	0.000
Duraphat - NUPRO White	-21.09	0.325	41.32	0.001	20.87	0.370
Duraphat - Prevident	-68.95	0.000	19.95	0.394	-11.15	0.925
Duraphat - ProFluorid	-4.38	0.999	48.53	0.000	10.49	0.944
Duraphat - Vanish	-30.42	0.033	62.83	0.000	11.16	0.925
Fluor Protector S - NUPRO White	-1.51	1.000	48.34	0.000	64.67	0.000
Fluor Protector S - Prevident	-49.37	0.000	26.97	0.089	32.65	0.022
Fluor Protector S - ProFluorid	15.20	0.714	55.55	0.000	54.29	0.000
Fluor Protector S - Vanish	-10.84	0.926	69.85	0.000	54.96	0.000
NUPRO White - Prevident	-47.86	0.000	-21.37	0.309	-32.02	0.026
NUPRO White - ProFluorid	16.71	0.614	7.21	0.990	-10.38	0.946
NUPRO White - Vanish	-9.33	0.964	21.51	0.301	-9.71	0.961
Prevident - ProFluorid	64.57	0.000	28.58	0.057	21.64	0.325
Prevident - Vanish	38.54	0.002	42.88	0.000	22.31	0.288
ProFluorid - Vanish	-26.03	0.113	14.30	0.769	0.67	1.000

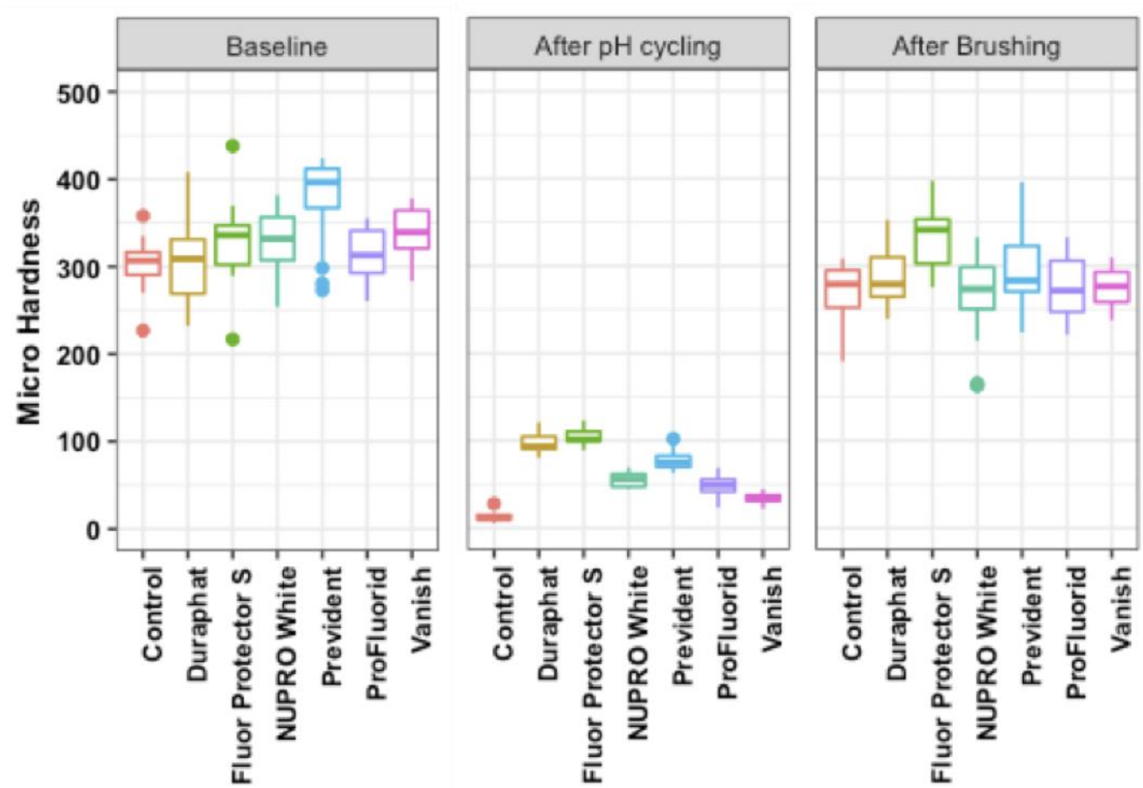


Figure 18. Boxplot of microhardness by varnish and time

Table 4. Descriptive statistics for tooth-wear

Varnish	Time	N	Mean	SD	Min	Max
Control	1 month brushing	19	51.28	10.96	34.67	73.33
Control	3 months brushing	19	64.72	13.49	41.67	90.67
Duraphat	1 month brushing	19	26.32	6.07	16.00	39.33
Duraphat	3 months brushing	19	33.28	5.53	24.67	47.67
Fluor Protector S	1 month brushing	19	27.16	5.08	15.33	39.00
Fluor Protector S	3 months brushing	19	33.42	5.13	22.67	42.00
NUPRO White	1 month brushing	19	32.88	9.34	22.00	62.67
NUPRO White	3 months brushing	19	40.02	11.37	28.00	77.00
Prevident	1 month brushing	19	37.05	9.50	26.67	58.00
Prevident	3 months brushing	19	45.00	8.96	33.67	64.67
ProFluorid	1 month brushing	19	44.44	13.63	31.00	85.33
ProFluorid	3 months brushing	19	51.79	13.83	39.00	94.33
Vanish	1 month brushing	19	36.82	9.07	26.00	60.00
Vanish	3 months brushing	19	44.74	10.45	30.67	70.33

Table 5. Pairwise comparisons for tooth-wear

	1-Month Brushing Mean Difference	P.Value	3-Month Brushing Mean Difference	P.Value
Control - Duraphat	24.96	0.000	31.44	0.000
Control - Fluor Protector S	24.12	0.000	31.30	0.000
Control - NUPRO White	18.40	0.000	24.70	0.000
Control - Prevident	14.23	0.000	19.72	0.000
Control - ProFluorid	6.84	0.341	12.93	0.001
Control - Vanish	14.46	0.000	19.98	0.000
Duraphat - Fluor Protector S	-0.84	1.000	-0.14	1.000
Duraphat - NUPRO White	-6.56	0.393	-6.74	0.360
Duraphat - Prevident	-10.74	0.017	-11.72	0.006
Duraphat - ProFluorid	-18.12	0.000	-18.51	0.000
Duraphat - Vanish	-10.51	0.021	-11.46	0.008
Fluor Protector S - NUPRO White	-5.72	0.565	-6.60	0.386
Fluor Protector S - Prevident	-9.89	0.037	-11.58	0.007
Fluor Protector S - ProFluorid	-17.28	0.000	-18.37	0.000
Fluor Protector S - Vanish	-9.67	0.046	-11.32	0.009
NUPRO White - Prevident	-4.18	0.853	-4.98	0.715
NUPRO White - ProFluorid	-11.56	0.007	-11.77	0.006
NUPRO White - Vanish	-3.95	0.883	-4.72	0.764
Prevident - ProFluorid	-7.39	0.251	-6.79	0.350
Prevident - Vanish	0.23	1.000	0.26	1.000
ProFluorid - Vanish	7.61	0.218	7.05	0.304

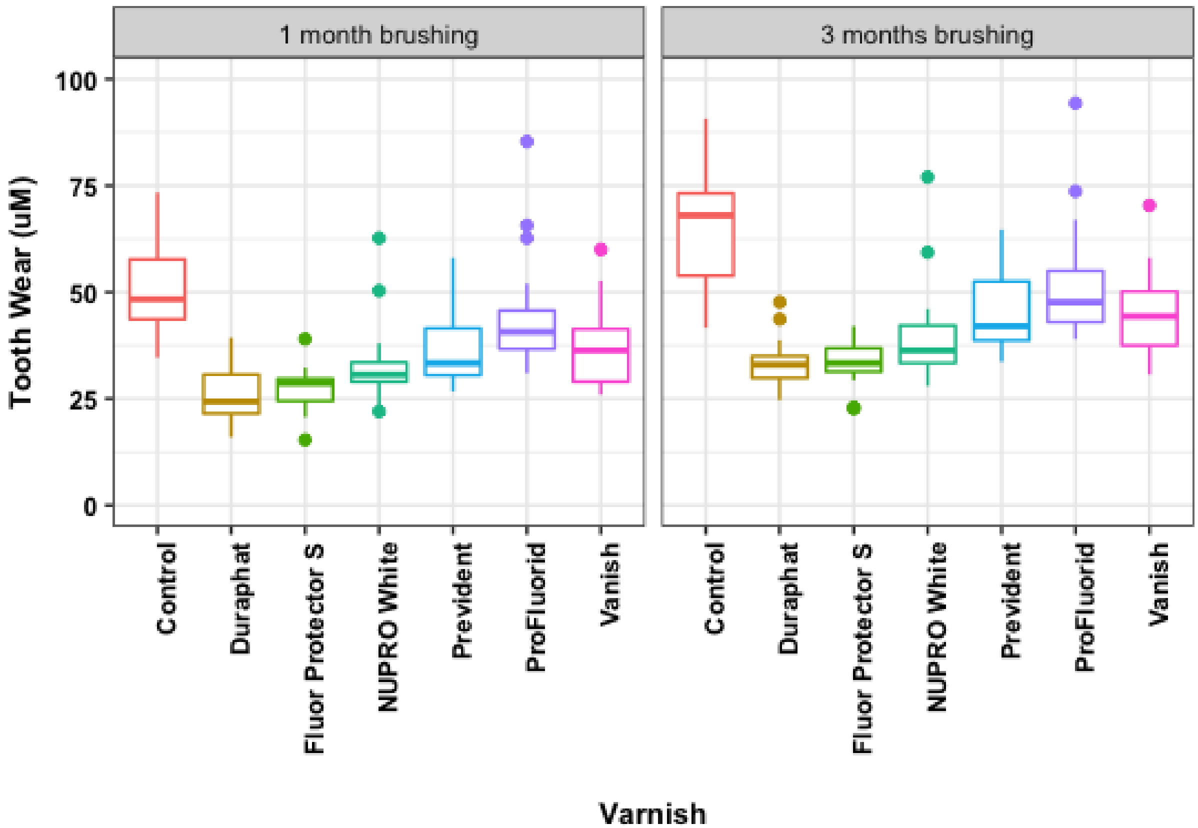


Figure 19: Boxplot of tooth-wear by varnish and time

3.3 Elemental analysis of the sample (EDS)

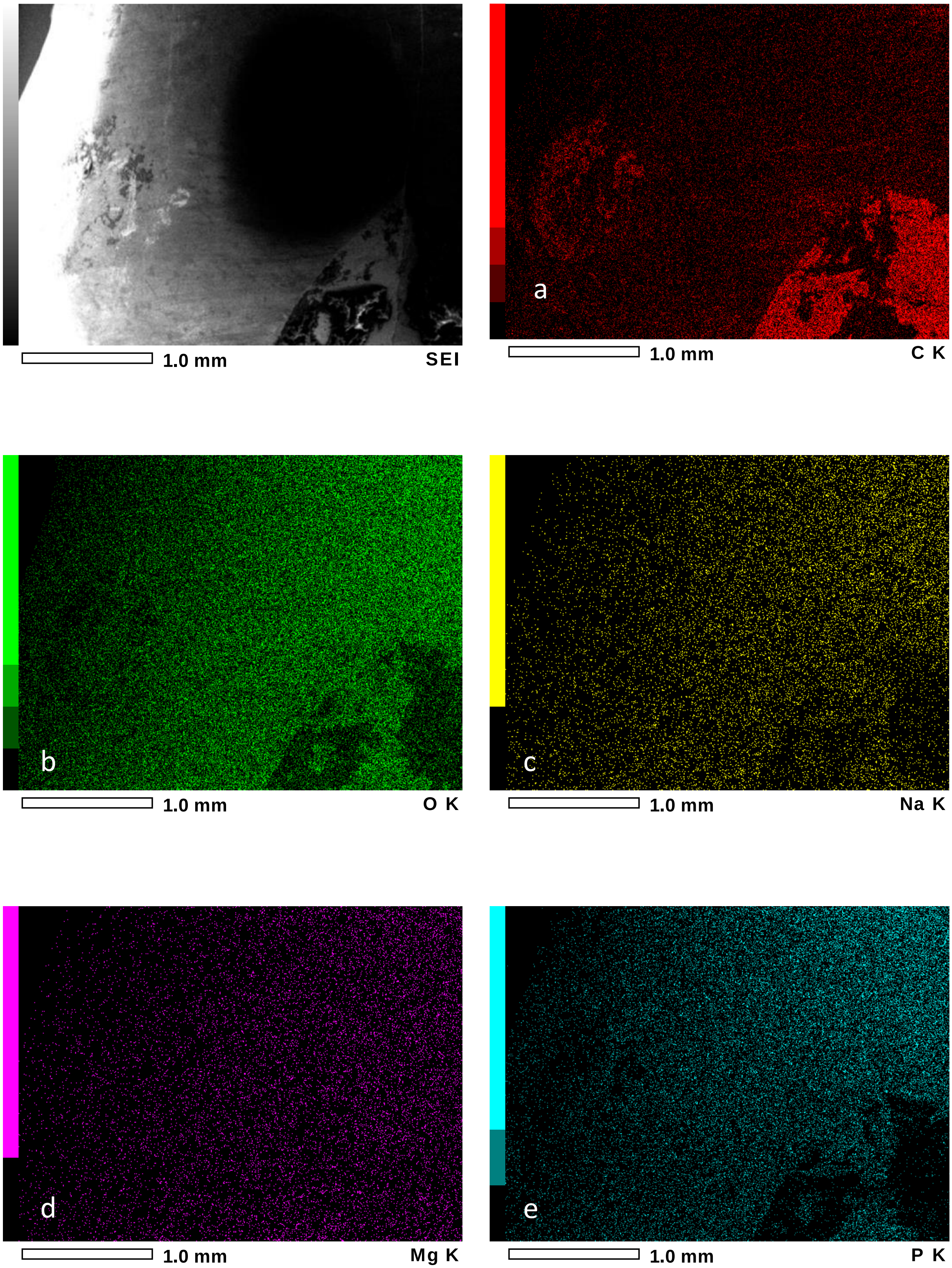
The EDS elemental analysis highlighted the presence of Ca and F ions in all treated specimens. After collecting EDS spectrum, an automatic identification of items is performed. Elemental analysis did not show fluoride on the surface of enamel for the baseline sample. While qualitative analysis revealed slight fluoride presence on the surface of the pH cycling sample, quantitative analysis was unable to detect fluoride as it was below the detection level.

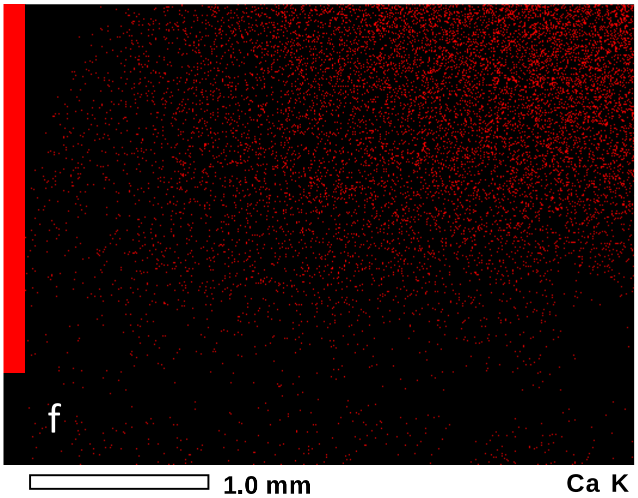
After brushing, Duraphat and Fluor Protector S samples have exhibited the highest amount of fluoride on the surface, showing 2.97%(mass) and 2.71%(mass) respectively. Fluoride was detected on the surface of Vanish group at 1.92%(mass). Control group has displayed the lowest amount of fluoride out of all groups at 1.3%(mass). The secondary electrons were recorded and they gave the topographical location of each element detected. Quantitative and qualitative results following EDS analysis of the specimens are detailed below (Figures 20-25) (Tables 6-11).

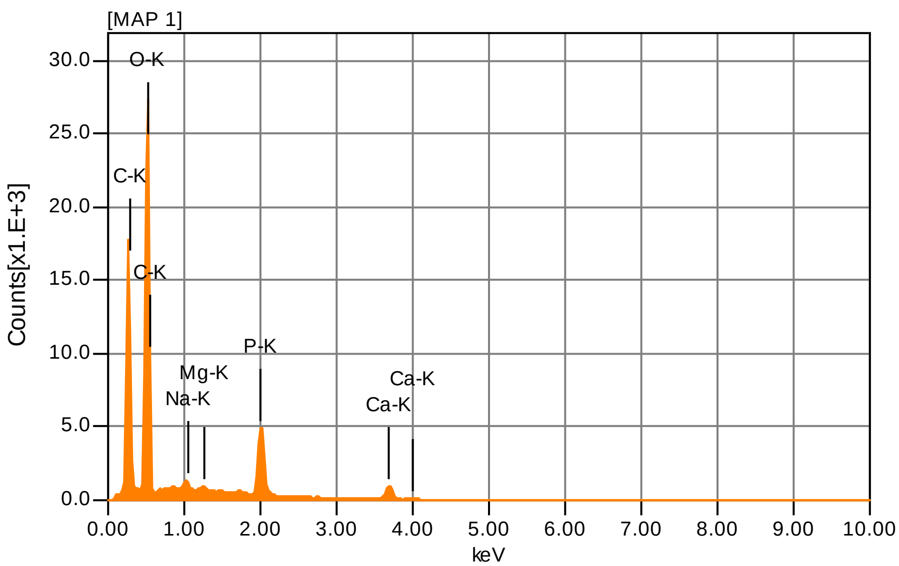
3.4 Scanning electron microscope

Morphological changes on the enamel surface after application of fluoride in SEM revealed the presence of globular precipitate in treated samples. Amorphous globular and crystalline structures were seen on the enamel surface. The specimen's surface was not covered completely by the globular precipitates. The globular precipitates were spherical in shape in treated samples (Figures 26-31).

MAP 1







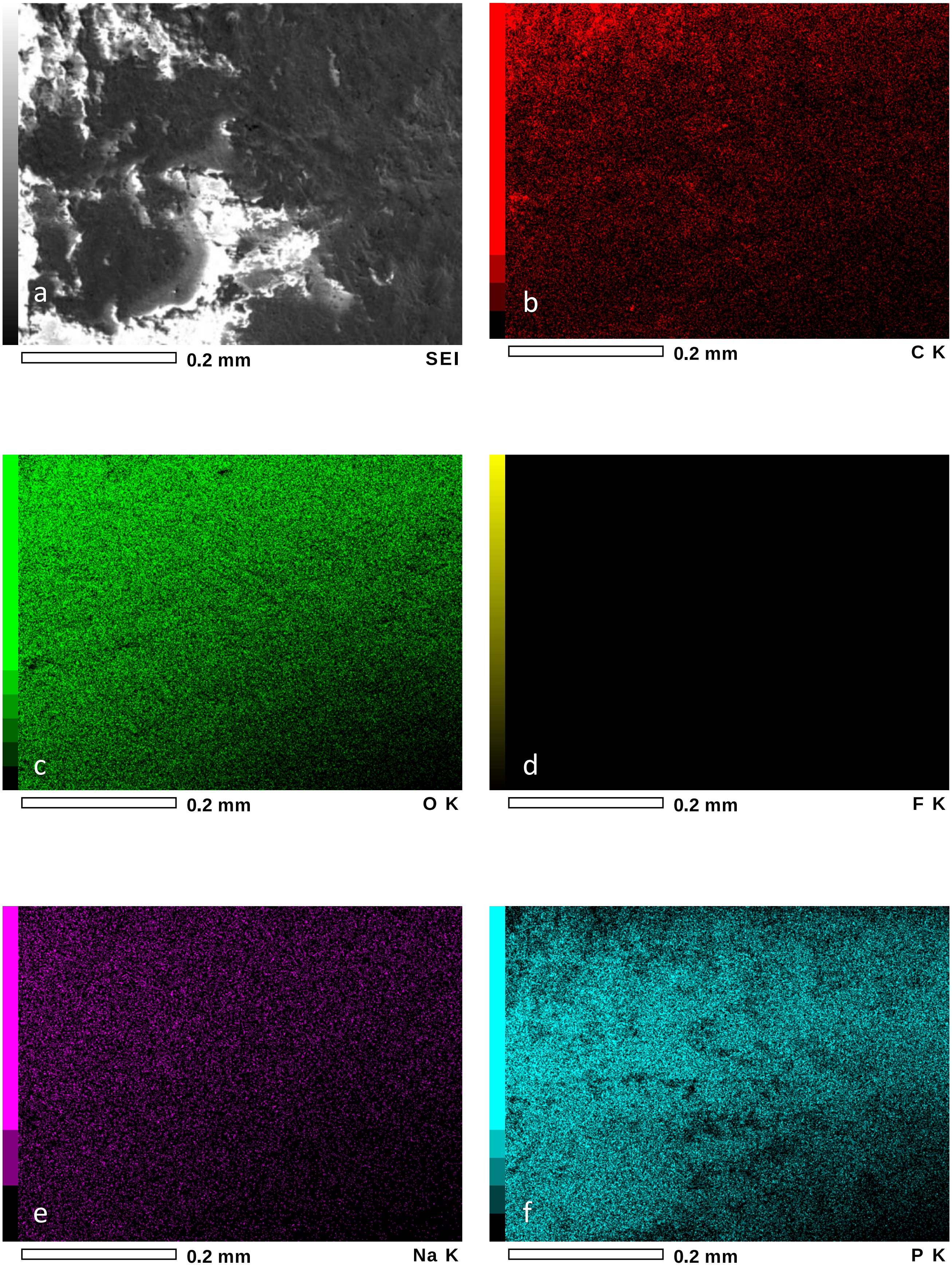
Acquisition Condition
Instrument : 6010LA
Volt : 15.00 kV
Current : ---
Process Time : T4
Live time : 188.82 sec.
Real Time : 196.60 sec.
DeadTime : 4.00 %
Count Rate : 2610.00 CPS

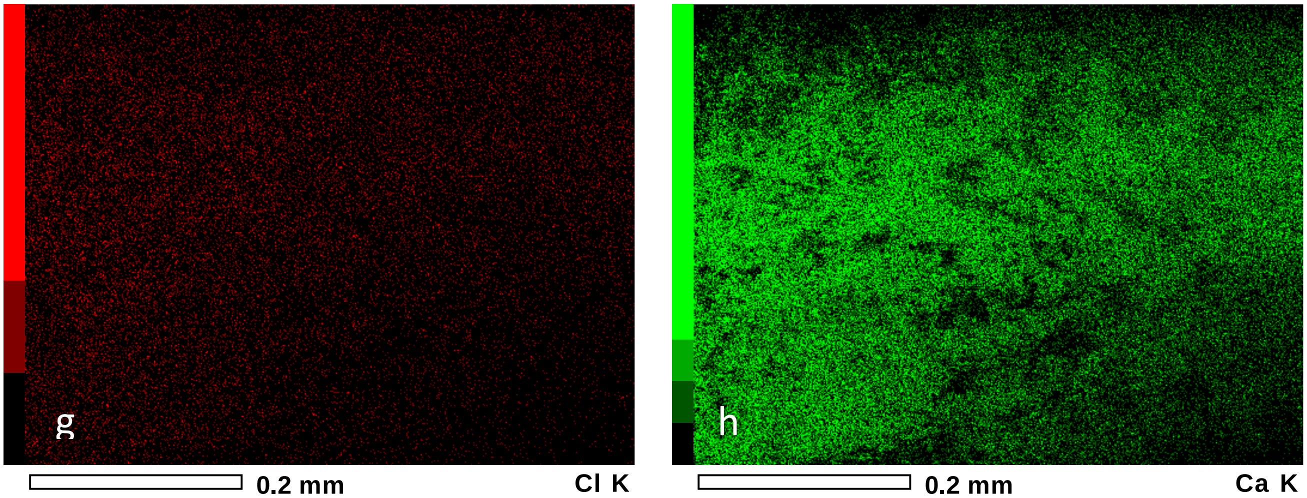
Formula	mass%	mol%	Cation	Sigma	Net	K ratio	Line
C	74.80	96.21	0.00	0.03	389920	0.0454165	K
O							
Na2O	1.14	0.28	1.12	0.02	18896	0.0046197	K
MgO	0.67	0.26	0.51	0.02	10976	0.0021703	K
P2O5	19.16	2.09	8.25	0.06	210536	0.0575277	K
CaO	4.23	1.17	2.31	0.03	49979	0.0236055	K
Total	100.00	100.00	12.18				

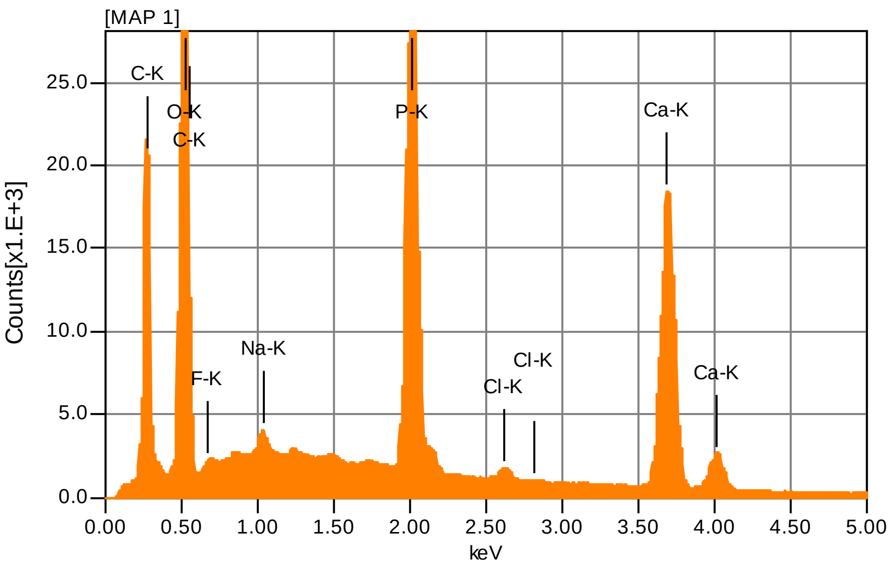
Figure 20: EDS qualitative analysis for baseline specimen showing topographical location of a) carbon, b) oxygen, c) sodium, d) magnesium, e) phosphate, and f) calcium ions.

Table 6: EDS automatic identification of items, and quantification of each item for baseline specimen.

MAP 1







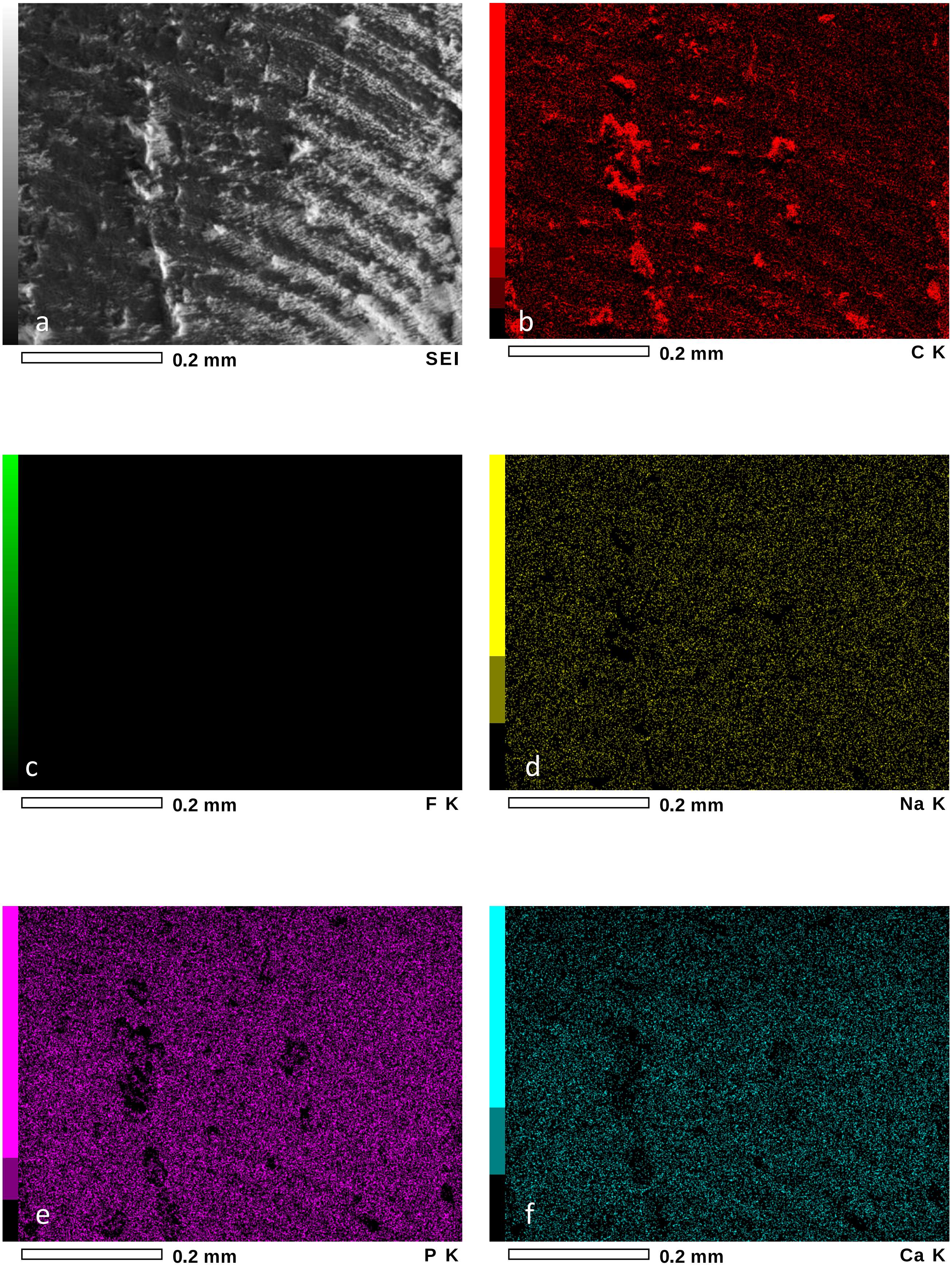
Acquisition Condition
Instrument : 6010LA
Volt : 15.00 kV
Current : ---
Process Time : T4
Live time : 163.25 sec.
Real Time : 196.60 sec.
DeadTime : 10.00 %
Count Rate : 4406.00 CPS

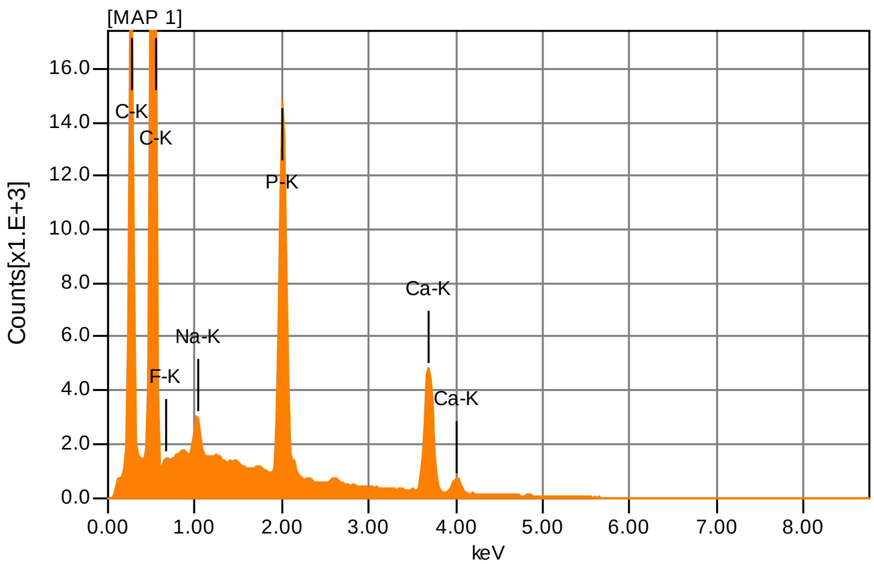
Formula	mass%	Atom%	Sigma	Net	K ratio	Line	Type
C	56.85	79.23	0.02	472178	0.0636120	K	
O				1727529	0.7627421	K	
F	nd	nd				K	
Na	0.69	0.50	0.01	45514	0.0128704	K	
P	20.36	11.00	0.02	1527722	0.4828243	K	
Cl	0.51	0.24	0.01	33799	0.0119628	K	
Ca	21.59	9.02	0.03	1041579	0.5689962	K	
Total	100.00	100.00					

Figure 21: a) SEM image of area selected for EDS analysis. EDS qualitative analysis for pH cycling specimen showing topographical location of b) carbon, c) oxygen, d) fluoride, e) sodium, f) phosphate, g) chloride, and h) calcium ions.

Table 7: EDS automatic identification of items, and quantification of each item for pH cycling specimen.

MAP 1





Acquisition Condition

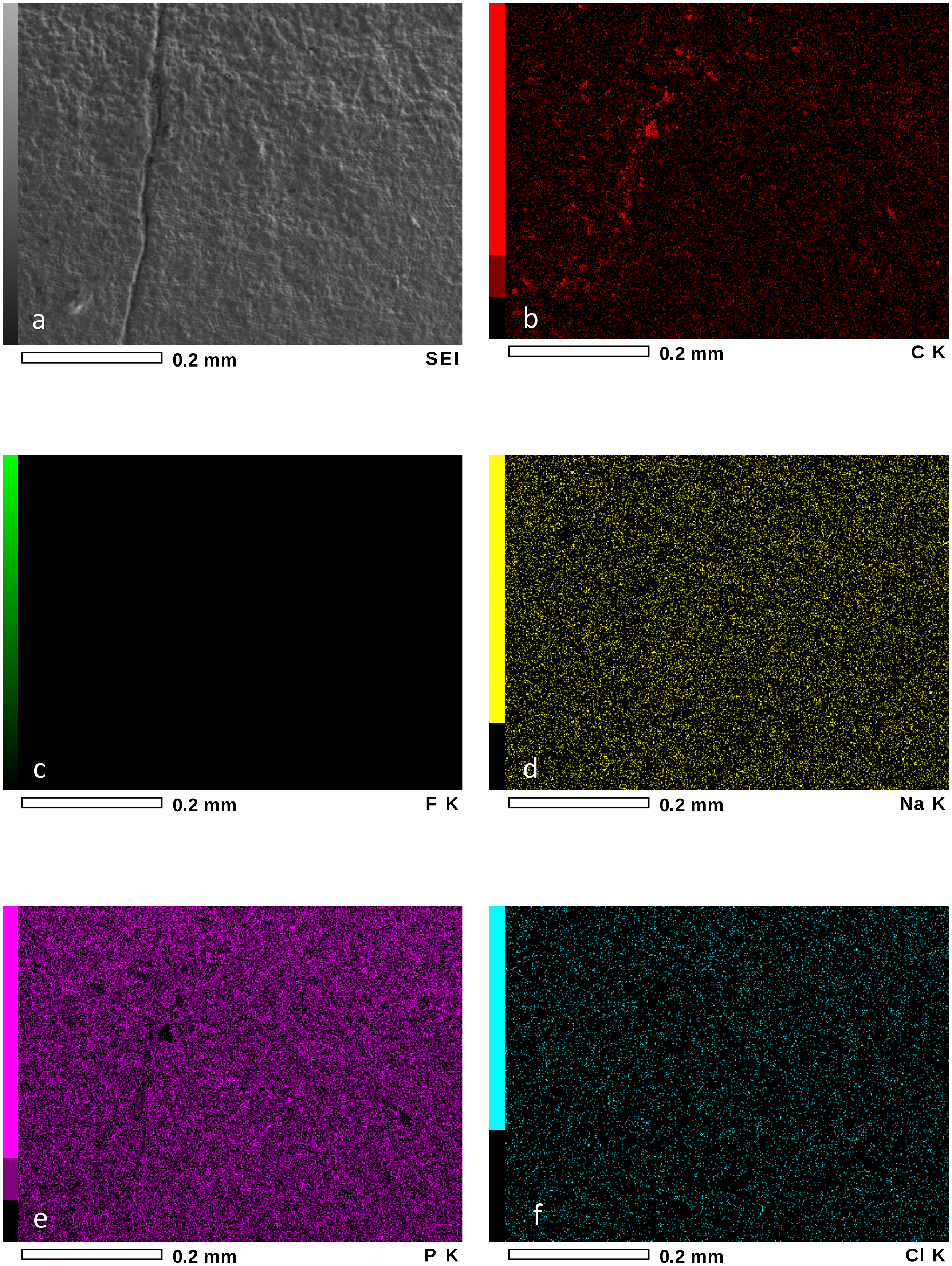
Instrument	:	6010LA
Volt	:	15.00 kV
Current	:	---
Process Time	:	T4
Live time	:	179.80 sec.
Real Time	:	196.60 sec.
DeadTime	:	7.00 %
Count Rate	:	5130.00 CPS

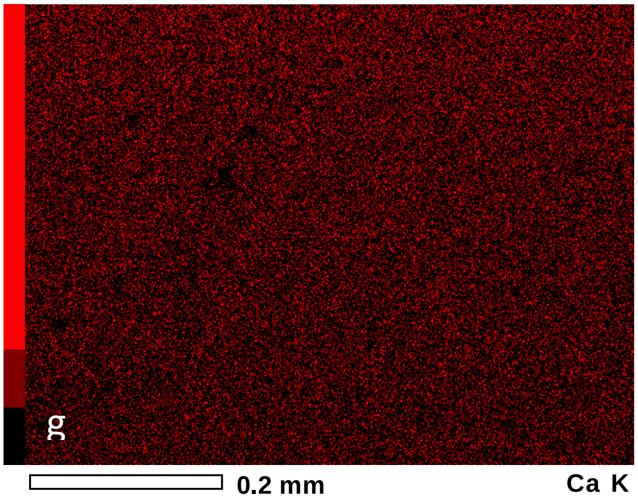
Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	73.32	87.55	0.02	518208	0.0633870	K
F*	2.97	2.24	0.02	36629	0.0284176	K
Na	1.06	0.66	0.01	43887	0.0112678	K
P	13.72	6.35	0.02	631660	0.1812556	K
Ca	8.93	3.20	0.03	265989	0.1319303	K
Total	100.00	100.00				

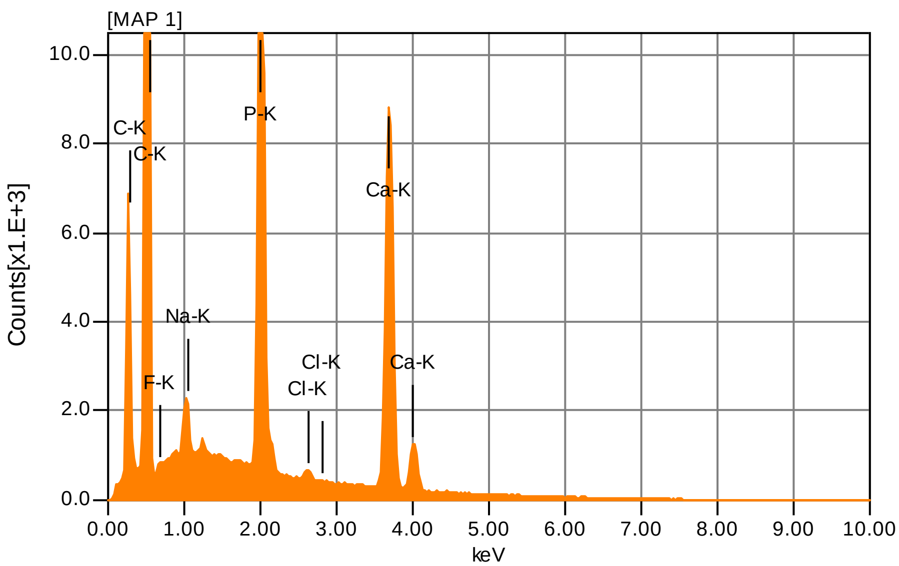
Figure 22: a) SEM image of area selected for EDS analysis. EDS qualitative analysis for Duraphat specimen showing topographical location of b) carbon, c) fluoride, d) sodium, e) phosphate, and f) calcium ions.

Table 8: EDS automatic identification of items, and quantification of each item for Duraphat specimen.

MAP 1







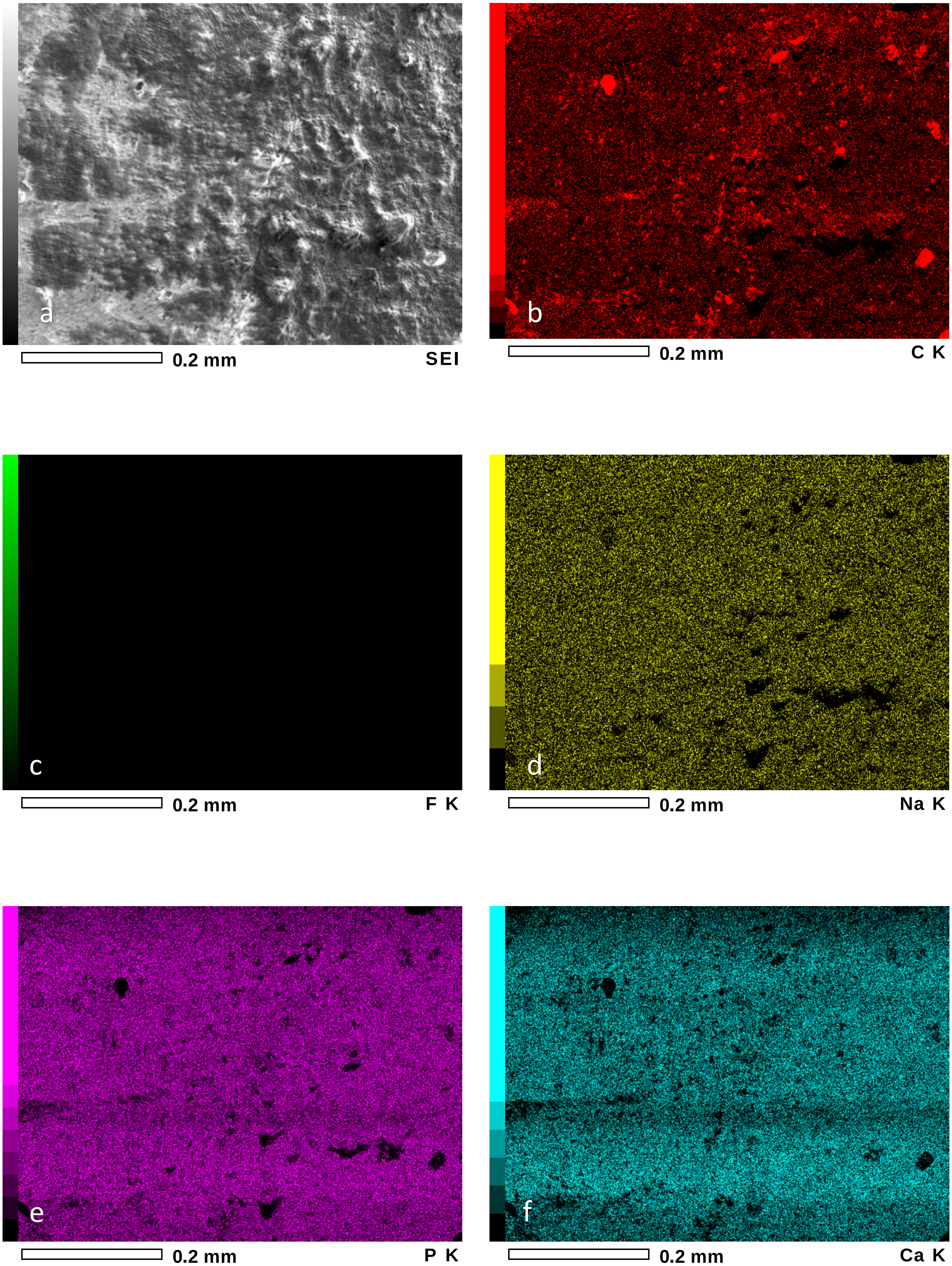

```
Acquisition Condition
Instrument      : 6010LA
Volt            : 15.00 kV
Current         : ---
Process Time    : T4
Live time       : 182.67
sec.
Real Time       : 196.60
sec.
DeadTime        : 7.00 %
Count Rate      : 4080.00
CPS
```

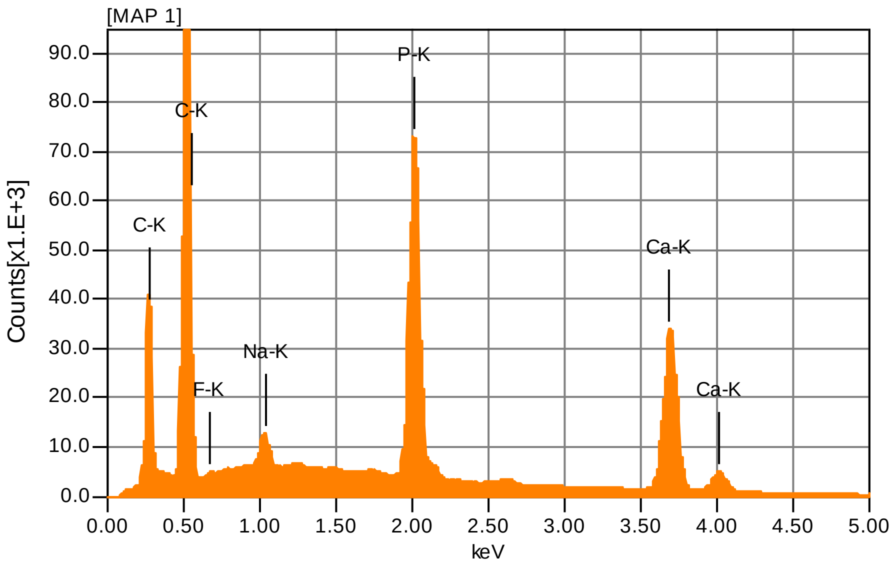
Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	47.67	71.56	0.03	147235	0.0177267	K
F*	2.71	2.57	0.03	20848	0.0159200	K
Na	1.52	1.19	0.02	40411	0.0102123	K
P	22.76	13.25	0.04	699611	0.1976002	K
Cl	0.38	0.19	0.01	10180	0.0032201	K
Ca	24.96	11.23	0.05	496991	0.2426339	K
Total	100.00	100.00				

Figure 23:a) SEM image of area selected for EDS analysis. EDS qualitative analysis for Fluor Protector S specimen showing topographical location of b) carbon, c) fluoride, d) sodium, e) phosphate, f) chloride, and g) calcium ions.

Table 9: EDS automatic identification of items, and quantification of each item for Fluor Protector S specimen.

MAP 1





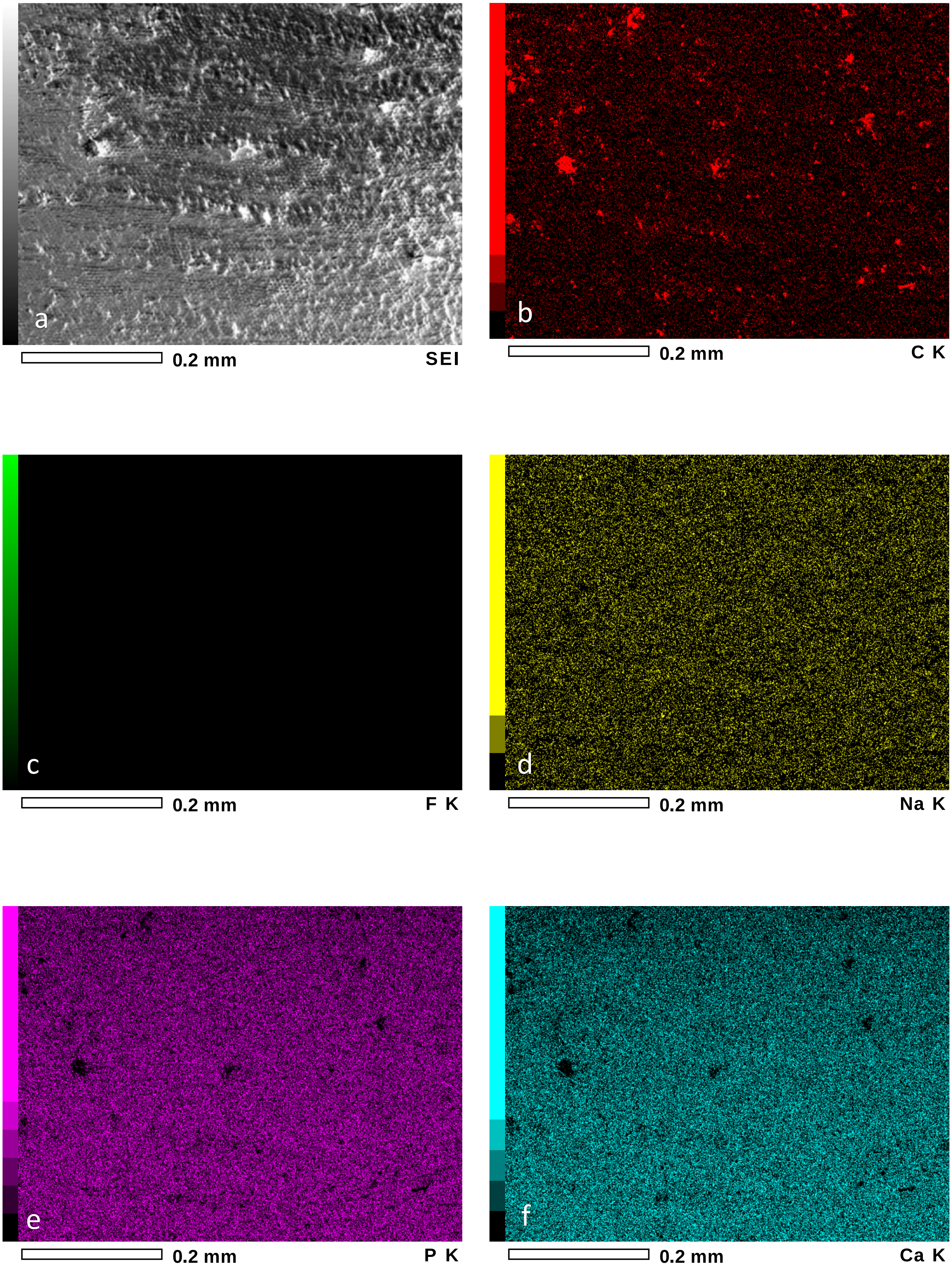

```
Acquisition Condition
Instrument       : 6010LA
Volt             : 10.00 kV
Current          : ---
Process Time     : T4
Live time        : 109.79
sec.
Real Time        : 196.60
sec.
DeadTime         : 39.00 %
Count Rate       : 30494.00
CPS
```

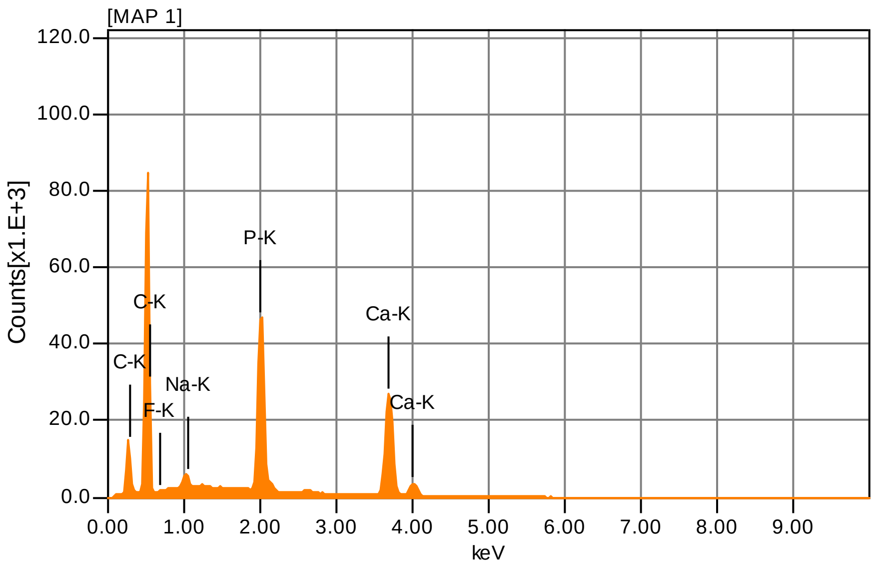
Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	32.91	58.29	0.01	880959	0.1931067	K
F*	1.92	2.15	0.01	114443	0.1285322	K
Na	1.53	1.41	0.01	214463	0.1245396	K
P	28.06	19.27	0.02	3064011	2.3873751	K
Ca	35.57	18.88	0.04	1845076	3.9085493	K
Total	100.00	100.00				

Figure 24: a) SEM image of area selected for EDS analysis. EDS qualitative analysis for Vanish specimen showing topographical location of b) carbon, c) fluoride, d) sodium, e) phosphate, f) chloride, and f) calcium ions.

Table 10: EDS automatic identification of items, and quantification of each item for Vanish specimen.

MAP 1





Acquisition Condition
Instrument : 6010LA
Volt : 10.00 kV
Current : ---
Process Time : T4
Live time : 156.95 sec.
Real Time : 196.60 sec.
DeadTime : 20.00 %
Count Rate : 13134.00 CPS

Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	20.49	42.98	0.01	317482	0.0486813	K
F*	1.30	1.72	0.01	44519	0.0349755	K
Na	1.20	1.31	0.01	98644	0.0400707	K
P	30.18	24.55	0.03	1974787	1.0763463	K
Ca	46.83	29.44	0.06	1463565	2.1687756	K
Total	100.00	100.00				

Figure 25: a) SEM image of area selected for EDS analysis. EDS qualitative analysis for control specimen showing topographical location of b) carbon, c) fluoride, d) sodium, e) phosphate, f) chloride, and f) calcium ions.

Table 11: EDS automatic identification of items, and quantification of each item for control specimen.

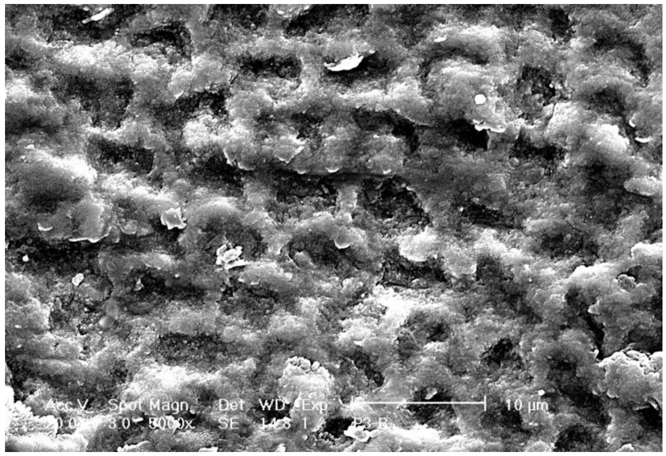


Figure 26: SEM image of ProFluorid sample at 5,000x magnification showing remaining varnish on enamel surface

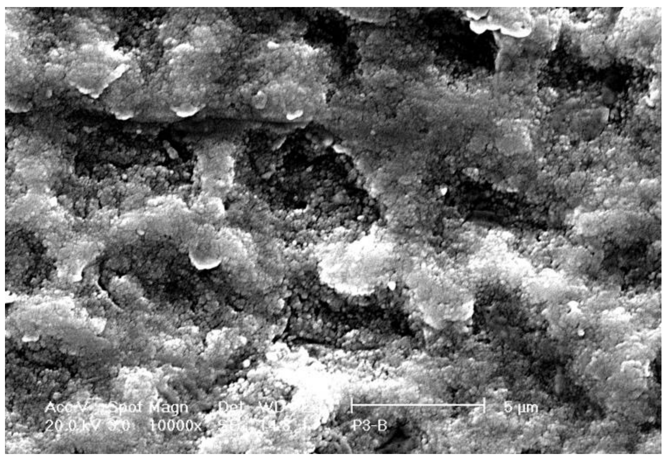


Figure 27: SEM image of ProFluorid sample at 10,000x magnification showing remaining varnish on enamel surface

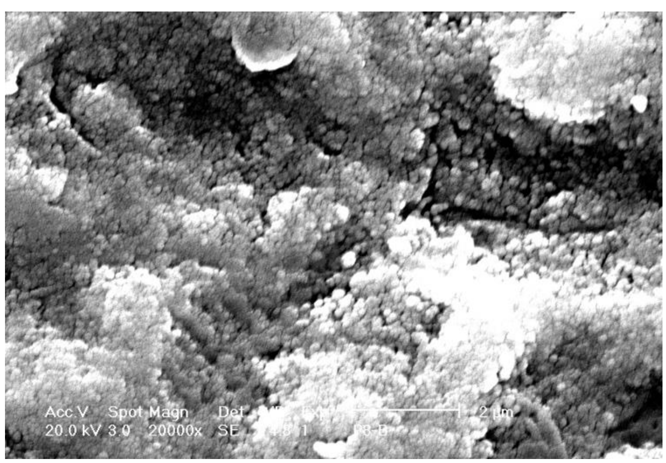


Figure 28: SEM image of the ProFluorid sample at 20,000x magnification showing remaining varnish. Presence of globular precipitates can be observed on the surface of the enamel

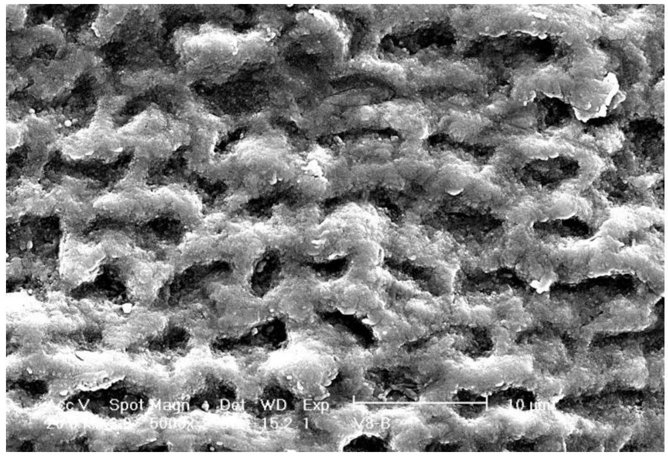


Figure 29: SEM image of Vanish sample at 5,000x magnification showing remaining varnish on enamel surface

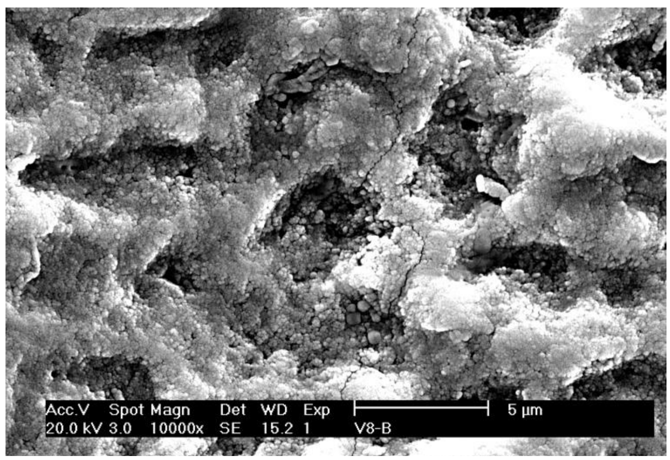


Figure 30: SEM image of Vanish sample at 10,000x magnification showing remaining varnish on enamel surface

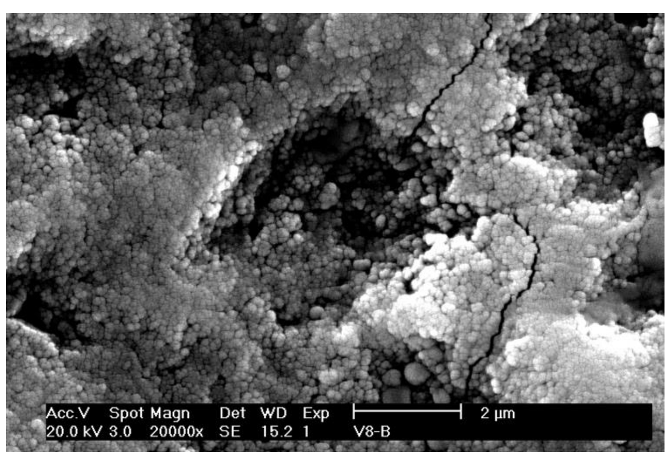


Figure 31: SEM image of the Vanish sample at 20,000x magnification showing remaining varnish. Presence of globular precipitates can be observed on the surface of the enamel

Chapter 4: Discussion

Previous research on fluoride effects shows that fluoride has a significant effect on the physical properties of enamel, mainly observed as changes in the surface microhardness of enamel.[58, 68] Several studies have shown that the surface microhardness of enamel improves when fluoride is applied to the enamel.[58, 69] Some studies show improvement in SMH of enamel when fluoride is applied with the intent of remineralization of the surface.[63, 70] This coincides with the results obtained from this study. However, to the author's best judgment, no studies investigating the resistance of enamel to wear after the application of fluoride varnish exists, which makes comparison of our findings to other studies challenging.

Control group has shown the least resistance to wear, as well as poor SMH when compared to the varnish groups, which leads us to accept the hypothesis that fluoride varnish, when applied to the enamel surface, promotes improvement in the physical properties of enamel, which in turn aids in the resistance to demineralization and toothbrush abrasion. However, the SMH of all groups was significantly affected to varying degrees depending on the type of fluoride varnish used. Between all groups, Fluor Protector S is the only group to show higher average SMH when compared to its' baseline. This may be due to the lower viscosity of the material, which might have enabled it to penetrate deeper into the enamel, thus creating stronger underlying enamel when compared to the other groups. Another factor could be the higher fluoride concentration of the varnish after drying, which enabled it to deliver fluoride more efficiently. Nevertheless, the material shows promising results, and should be investigated further in future studies.

The amount of wear observed on all groups shows that most of the demineralized superficial enamel is lost within the first month of brushing when compared to three months of brushing. Furthermore, based on careful review of the literature, our results show that demineralized enamel is more readily lost than intact enamel, which requires years of brushing to lose a significant amount of the surface.

Although five varnishes used in this study share the same active ingredient of fluoride (NaF), results show significant difference between these groups. This leads to the conclusion that different formulations significantly affect the function of the varnishes. Furthermore, the new ammonium fluoride varnish has shown the least amount of surface loss when compared to the other groups, and showing similar results to Duraphat varnish. Careful understanding of this material is recommended, and future wear studies should be conducted to test the extent of the protective effect of this varnish. Control group showed the most surface loss when compared to the varnish groups, thus our hypothesis is accepted. It is worth noting that the Vanish group, which showed the second least SMH, showed similar wear resistance to two other groups of varnish (NUPRO White and PreviDent) which showed better SMH. Such observation leads to the assumption that reduced SMH does not always lead to more wear. However, significant wear was still observed.

SEM has been used to evaluate the remineralization effect of fluoride on enamel by several authors.[71-73] Specimens are coated with metals such as gold and palladium to improve image quality in most studies.[71, 73] In our study, we used palladium coating since it does not interfere with the accuracy of EDS analysis compared to gold. After treating the enamel with fluoride varnish, globular and amorphous precipitates were detected on the surface, and we can assume that this precipitation will provide a reservoir of fluoride which would extend the caries preventive effect of the material for longer periods. Elemental analysis supports this observation, as the treated samples had a higher percentage of fluoride on the surface. Earlier studies have also described the presence of globular material on the enamel after contact with topical fluorides.[72, 73] While past studies were able to detect fluoride ions at higher percentage, our study aimed to evaluate the retention of the fluoride varnish after three months of toothbrushing, which led to the low percentage of fluoride observed. The presence of fluoride in the control group could be attributed to the source of the tooth. Different teeth from different sources are expected to show varying percentages of fluoride content at baseline.

The results obtained from this study show that while fluoride varnish has shown significant protective ability on human enamel when compared to the control group, differences were detected within the different groups. This observation provides necessary knowledge needed to further understand the protective potential of fluoride varnishes specifically, and all topical fluorides in general. The effect of toothbrushing on demineralized enamel is significant, and understanding this fact is important for the dental practitioner. Careful management of high caries risk patients should include educating the patient of the risks involved with toothbrushing, and the abrasivity of the toothpastes they use. Another factor is the time of toothbrushing. Care should be taken to educate the patients to avoid brushing immediately after consuming acidic foods and drinks, as that would lead to more damage to the enamel surface. More studies of this kind should be conducted in the future, possibly with different fluoride varnish formulations, to evaluate the extent of protection fluoride provides for enamel, as well as the effect of different formulations with the same active ingredient on remineralization, SMH, and wear resistance of enamel.

Intraoral scanners proved to be a logical and efficient alternative to the more commonly used methods to quantify tooth wear. Accessibility and availability of digital scanners makes them a more efficient choice when compared to the more expensive and narrow application instruments such as profilometers and laser 3D scans. While different software were used for the superimposition of scans, as well as wear measurements,[49, 50] exocad proves to be a suitable instrument for measuring wear. The most obvious limitation of these software is their inability to quantify the volumetric loss of tooth structure. Nevertheless, future studies of this kind should aim to employ methods that are able to quantify the volumetric loss of tooth structure.

Chapter 5: Conclusion

Within the limitations of this study, fluoride varnishes have displayed a significant protective effect on the enamel surface. However, not all fluoride varnishes have performed equally, despite most of them sharing the same active ingredient. Results from this study shows that globular precipitations on the surface of enamel are observed after three-months brushing on treated samples, which indicated effectiveness of fluoride source for longer periods. Said effectiveness has led to decreased loss of enamel surface after long term brushing simulation (three-months). The innovative approach of surface wear assessment using intra-oral scanner and specialized software provides vital information for monitoring the progression of tooth surface loss.

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Appendix A. Raw data for microhardness measurements

Sample	Varnish (Group)	Time	Microhardness 1	Microhardness 2	Microhardness 3	AVG Microhardness
F1	Fluor Protector S	Baseline	256.1	320.6	299.1	291.9333333
F2	Fluor Protector S	Baseline	333	358.3	346	345.7666667
F3	Fluor Protector S	Baseline	393.8	349	366.3	369.7
F4	Fluor Protector S	Baseline	367.9	341.6	343.1	350.8666667
F5	Fluor Protector S	Baseline	335.8	359.9	364.7	353.4666667
F6	Fluor Protector S	Baseline	363.1	350.5	324.6	346.0666667
F7	Fluor Protector S	Baseline	367.9	305.2	379.7	350.9333333
F8	Fluor Protector S	Baseline	315.3	353.6	323	330.6333333
F9	Fluor Protector S	Baseline	459	463.6	392	438.2
F10	Fluor Protector S	Baseline	349	327.4	327.4	334.6
F11	Fluor Protector S	Baseline	295.5	299.1	302.7	299.1
F12	Fluor Protector S	Baseline	274.3	288.6	306.4	289.7666667
F13	Fluor Protector S	Baseline	293.2	295.5	319.3	302.6666667
F14	Fluor Protector S	Baseline	318	346	349	337.6666667
F15	Fluor Protector S	Baseline	334.4	356.7	341.6	344.2333333
F16	Fluor Protector S	Baseline	307.7	327.4	305.2	313.4333333
F17	Fluor Protector S	Baseline	284.1	323.3	300.3	302.5666667
F18	Fluor Protector S	Baseline	328.8	343.1	359.9	343.9333333
F19	Fluor Protector S	Baseline	215.3	199.3	236.5	217.0333333
F20	Fluor Protector S	Baseline	283	320.6	301.5	301.7
F1	Fluor Protector S	After pH cycling	101.7	117.7	120.7	113.3666667
F2	Fluor Protector S	After pH cycling	120	95.2	115.8	110.3333333
F3	Fluor Protector S	After pH cycling	103.2	98	100.3	100.5
F4	Fluor Protector S	After pH cycling	93.4	101.3	106.2	100.3
F5	Fluor Protector S	After pH cycling	93.2	105.1	103.6	100.6333333
F6	Fluor Protector S	After pH cycling	134	97.1	90.5	107.2
F7	Fluor Protector S	After pH cycling	103.4	91.7	100.7	98.6
F8	Fluor Protector S	After pH cycling	120	128.6	121.3	123.3
F9	Fluor Protector S	After pH cycling	118.3	125.8	110.9	118.3333333
F10	Fluor Protector S	After pH cycling	101.2	108.2	94.9	101.4333333
F11	Fluor Protector S	After pH cycling	98.5	91.2	78.2	89.3

F12	Fluor Protector S	After pH cycling	100.7	104.4	127.2	110.7666667
F13	Fluor Protector S	After pH cycling	87.3	93.6	89.2	90.03333333
F14	Fluor Protector S	After pH cycling	102.2	101.2	92	98.46666667
F15	Fluor Protector S	After pH cycling	119.5	105.1	102.7	109.1
F16	Fluor Protector S	After pH cycling	100.8	105	99.4	101.7333333
F17	Fluor Protector S	After pH cycling	108.2	120.7	115.9	114.9333333
F18	Fluor Protector S	After pH cycling	97.6	93.6	111.4	100.8666667
F19	Fluor Protector S	After pH cycling	112.6	104.1	96.7	104.4666667
F20	Fluor Protector S	After pH cycling	91.6	90	93.2	91.6
F1	Fluor Protector S	After Brushing	338.5	355.5	362.2	352.0666667
F2	Fluor Protector S	After Brushing	476.5	346.8	369.1	397.4666667
F3	Fluor Protector S	After Brushing	355.5	373.8	340.5	356.6
F4	Fluor Protector S	After Brushing	313.4	351.1	359.9	341.4666667
F5	Fluor Protector S	After Brushing	322.7	297.6	404	341.4333333
F6	Fluor Protector S	After Brushing	346.8	376.2	326.6	349.8666667
F7	Fluor Protector S	After Brushing	284.6	246.4	308	279.6666667
F8	Fluor Protector S	After Brushing	369.1	326.6	306.2	333.9666667
F9	Fluor Protector S	After Brushing	315.2	296	301	304.0666667
F10	Fluor Protector S	After Brushing	338.5	346.8	364.5	349.9333333
F11	Fluor Protector S	After Brushing	346.8	342.6	381	356.8
F12	Fluor Protector S	After Brushing				
F13	Fluor Protector S	After Brushing	294.3	353.3	308	318.5333333
F14	Fluor Protector S	After Brushing	315.2	332.5	256.8	301.5
F15	Fluor Protector S	After Brushing	359.9	359.9	315.2	345
F16	Fluor Protector S	After Brushing	230.6	357.7	238.9	275.7333333
F17	Fluor Protector S	After Brushing	246.4	309.8	273.9	276.7
F18	Fluor Protector S	After Brushing	391	326.6	346.8	354.8
F19	Fluor Protector S	After Brushing	315.2	412.1	366.8	364.7
F20	Fluor Protector S	After Brushing	278.4	266.6	330.5	291.8333333
V1	Vanish	Baseline	346	374.6	384.9	368.5
V2	Vanish	Baseline	374.6	372.9	386.6	378.0333333
V3	Vanish	Baseline	361.5	372.9	356.7	363.7
V4	Vanish	Baseline	378	324.6	331.5	344.7
V5	Vanish	Baseline	372.9	364.7	369.6	369.0666667
V6	Vanish	Baseline	335.8	338.7	347.5	340.6666667

V7	Vanish	Baseline	331.5	343.1	338.7	337.7666667
V8	Vanish	Baseline	384.9	367.9	353.6	368.8
V9	Vanish	Baseline	305.2	327.4	331.5	321.3666667
V10	Vanish	Baseline	347.5	352.1	323.3	340.9666667
V11	Vanish	Baseline	347.5	338.7	328.8	338.3333333
V12	Vanish	Baseline	307.7	392	399.3	366.3333333
V13	Vanish	Baseline	327.4	258.2	330.1	305.2333333
V14	Vanish	Baseline	289.7	295.5	301.5	295.5666667
V15	Vanish	Baseline	300.3	281.9	268.9	283.7
V16	Vanish	Baseline	328.8	327.4	331.5	329.2333333
V17	Vanish	Baseline	324.6	318	310.2	317.6
V18	Vanish	Baseline	312.8	330.1	316.6	319.8333333
V19	Vanish	Baseline	335.8	341.6	331.5	336.3
V20	Vanish	Baseline	334.4	353.6	378	355.3333333
V1	Vanish	After pH cycling	38.5	33.4	31.6	34.5
V2	Vanish	After pH cycling	24.6	36.9	40.3	33.93333333
V3	Vanish	After pH cycling	30.7	29.7	33	31.13333333
V4	Vanish	After pH cycling	33.2	44.3	33.4	36.96666667
V5	Vanish	After pH cycling	23.8	31.4	26.1	27.1
V6	Vanish	After pH cycling	36.5	37.4	40.7	38.2
V7	Vanish	After pH cycling	23.3	30.4	34.7	29.46666667
V8	Vanish	After pH cycling	32	30.2	39.3	33.83333333
V9	Vanish	After pH cycling	21.3	25	20.5	22.26666667
V10	Vanish	After pH cycling	45.7	31.4	26.1	34.4
V11	Vanish	After pH cycling	35.7	29	29.8	31.5
V12	Vanish	After pH cycling	43.6	30.9	41.2	38.56666667
V13	Vanish	After pH cycling	51.3	34.2	46	43.83333333
V14	Vanish	After pH cycling	40.2	40.1	39.9	40.06666667
V15	Vanish	After pH cycling	41.6	50	42.6	44.73333333
V16	Vanish	After pH cycling	38.8	35.3	38.5	37.53333333
V17	Vanish	After pH cycling	35.8	32.4	31.3	33.16666667
V18	Vanish	After pH cycling	29.9	29.5	32.7	30.7
V19	Vanish	After pH cycling	41.9	25.7	29.5	32.36666667
V20	Vanish	After pH cycling	35	32.7	34	33.9
V1	Vanish	After Brushing				
V2	Vanish	After Brushing	283	240.1	344.7	289.2666667

V3	Vanish	After Brushing	263.7	299.3	278.4	280.4666667
V4	Vanish	After Brushing	306.2	311.6	263.7	293.8333333
V5	Vanish	After Brushing	273.9	262.3	213.3	249.8333333
V6	Vanish	After Brushing	272.4	346.8	291	303.4
V7	Vanish	After Brushing	287.8	299.3	311.6	299.5666667
V8	Vanish	After Brushing	276.9	306.2	203.4	262.1666667
V9	Vanish	After Brushing	272.4	269.5	284.6	275.5
V10	Vanish	After Brushing	320.8	284.6	291	298.8
V11	Vanish	After Brushing	245.1	278.4	284.6	269.3666667
V12	Vanish	After Brushing	251.5	220.6	287.8	253.3
V13	Vanish	After Brushing	342.6	272.4	262.3	292.4333333
V14	Vanish	After Brushing	227.2	213.3	315.2	251.9
V15	Vanish	After Brushing	332.5	260.9	255.5	282.9666667
V16	Vanish	After Brushing	243.8	233	289.4	255.4
V17	Vanish	After Brushing	275.4	193.3	245.1	237.9333333
V18	Vanish	After Brushing	328.5	252.8	248.9	276.7333333
V19	Vanish	After Brushing	309.8	340.6	278.4	309.6
V20	Vanish	After Brushing	262.3	322.7	211.3	265.4333333
N1	NUPRO White	Baseline	397.5	376.3	371.2	381.6666667
N2	NUPRO White	Baseline	295.5	328.8	299.1	307.8
N3	NUPRO White	Baseline	381.4	358.3	395.6	378.4333333
N4	NUPRO White	Baseline	363.1	390.2	392	381.7666667
N5	NUPRO White	Baseline	378	335.8	334.4	349.4
N6	NUPRO White	Baseline	341.6	358.3	358.3	352.7333333
N7	NUPRO White	Baseline	343.1	338.7	369.6	350.4666667
N8	NUPRO White	Baseline	327.4	334.4	330.1	330.6333333
N9	NUPRO White	Baseline	378	381.4	350.5	369.9666667
N10	NUPRO White	Baseline	358.3	395.6	353.6	369.1666667
N11	NUPRO White	Baseline	277.5	275.4	271.2	274.7
N12	NUPRO White	Baseline	270.1	241.7	250.6	254.1333333
N13	NUPRO White	Baseline	312.8	327.4	341.6	327.2666667
N14	NUPRO White	Baseline	246.1	266.1	264.1	258.7666667
N15	NUPRO White	Baseline	302.7	295.5	321.9	306.7
N16	NUPRO White	Baseline	327.4	330.1	324.6	327.3666667
N17	NUPRO White	Baseline	305.2	323.3	302.7	310.4
N18	NUPRO White	Baseline	326	353.6	320.6	333.4
N19	NUPRO White	Baseline	312.8	271.2	283	289
N20	NUPRO White	Baseline	334.4	349	338.7	340.7
N1	NUPRO White	After pH cycling	74.5	60.2	59.1	64.6

N2	NUPRO White	After pH cycling	40.3	56.1	55.7	50.7
N3	NUPRO White	After pH cycling	45.1	54.7	40.3	46.7
N4	NUPRO White	After pH cycling	66.3	57.3	54.9	59.5
N5	NUPRO White	After pH cycling	67.8	67.3	73.3	69.46666667
N6	NUPRO White	After pH cycling	45.4	50.7	44.7	46.93333333
N7	NUPRO White	After pH cycling	45.8	41.6	53.3	46.9
N8	NUPRO White	After pH cycling	55.9	62.9	63.8	60.86666667
N9	NUPRO White	After pH cycling	71	61.1	67.8	66.63333333
N10	NUPRO White	After pH cycling	56.5	71	69.4	65.63333333
N11	NUPRO White	After pH cycling	51	69.9	82.2	67.7
N12	NUPRO White	After pH cycling	73.3	49.7	58.3	60.43333333
N13	NUPRO White	After pH cycling	56.1	42.7	44.6	47.8
N14	NUPRO White	After pH cycling	48.9	45	48.1	47.33333333
N15	NUPRO White	After pH cycling	46.7	54.9	50.7	50.76666667
N16	NUPRO White	After pH cycling	43.2	46.1	48.9	46.06666667
N17	NUPRO White	After pH cycling	44.6	47.5	56.5	49.53333333
N18	NUPRO White	After pH cycling	54.9	58.9	49	54.26666667
N19	NUPRO White	After pH cycling	64.3	54.9	56.5	58.56666667
N20	NUPRO White	After pH cycling	60.2	62.7	51	57.96666667
N1	NUPRO White	After Brushing	296	302.8	349	315.9333333
N2	NUPRO White	After Brushing	294.3	235.3	357.7	295.7666667
N3	NUPRO White	After Brushing	275.4	260.9	284.6	273.6333333
N4	NUPRO White	After Brushing				
N5	NUPRO White	After Brushing	320.8	291	254.2	288.6666667
N6	NUPRO White	After Brushing	231.8	259.6	376.2	289.2
N7	NUPRO White	After Brushing	278.4	319	313.4	303.6
N8	NUPRO White	After Brushing	308	296	301	301.6666667
N9	NUPRO White	After Brushing	344.7	304.5	324.6	324.6
N10	NUPRO White	After Brushing	304.5	328.5	364.5	332.5
N11	NUPRO White	After Brushing	208.3	177.6	278.4	221.4333333
N12	NUPRO White	After Brushing	156.4	123.5	217.4	165.7666667
N13	NUPRO White	After Brushing	167.9	266.6	308	247.5
N14	NUPRO White	After Brushing	228.4	168.6	247.6	214.8666667
N15	NUPRO White	After Brushing	173.8	162.3	152.6	162.9
N16	NUPRO White	After Brushing	219.6	218.5	322.7	253.6
N17	NUPRO White	After Brushing	279.9	273.9	258.2	270.6666667

N18	NUPRO White	After Brushing	301	279.9	251.5	277.4666667
N19	NUPRO White	After Brushing	291	231.8	281.5	268.1
N20	NUPRO White	After Brushing	230.6	265.1	270.9	255.5333333
PF1	ProFluorid	Baseline	289.7	305.2	338.7	311.2
PF2	ProFluorid	Baseline	363.1	353.6	341.6	352.7666667
PF3	ProFluorid	Baseline	344.5	346	341.6	344.0333333
PF4	ProFluorid	Baseline	292	297.9	290.9	293.6
PF5	ProFluorid	Baseline	307.7	346	334.4	329.3666667
PF6	ProFluorid	Baseline	320.6	319.3	343.1	327.6666667
PF7	ProFluorid	Baseline	338.7	331.5	395.6	355.2666667
PF8	ProFluorid	Baseline	327.4	376.3	359.9	354.5333333
PF9	ProFluorid	Baseline	350.5	328.8	341.6	340.3
PF10	ProFluorid	Baseline	308.9	335.8	318	320.9
PF11	ProFluorid	Baseline	255.3	278.6	257.2	263.7
PF12	ProFluorid	Baseline	285.2	304	310.2	299.8
PF13	ProFluorid	Baseline	285.2	287.4	297.9	290.1666667
PF14	ProFluorid	Baseline	359.9	343.1	334.4	345.8
PF15	ProFluorid	Baseline	261.1	239.1	281.9	260.7
PF16	ProFluorid	Baseline	293.2	283	295.5	290.5666667
PF17	ProFluorid	Baseline	315.3	314	316.6	315.3
PF18	ProFluorid	Baseline	308.9	293.2	286.3	296.1333333
PF19	ProFluorid	Baseline	275.4	269.1	256.3	266.9333333
PF20	ProFluorid	Baseline	295.5	310.2	299.1	301.6
PF1	ProFluorid	After pH cycling	38	41.4	38.6	39.33333333
PF2	ProFluorid	After pH cycling	37.1	29.8	38.8	35.23333333
PF3	ProFluorid	After pH cycling	31.4	32.4	29.3	31.03333333
PF4	ProFluorid	After pH cycling	44.4	43.1	31	39.5
PF5	ProFluorid	After pH cycling	42.6	29.3	75.4	49.1
PF6	ProFluorid	After pH cycling	58.7	64.1	69.1	63.96666667
PF7	ProFluorid	After pH cycling	45.4	54.7	47.6	49.23333333
PF8	ProFluorid	After pH cycling	63.4	51	71.3	61.9
PF9	ProFluorid	After pH cycling	73.9	69.4	64.1	69.13333333
PF10	ProFluorid	After pH cycling	58.3	55.3	52.9	55.5
PF11	ProFluorid	After pH cycling	57.1	56.1	51	54.73333333
PF12	ProFluorid	After pH cycling	22.4	24.3	24.7	23.8
PF13	ProFluorid	After pH cycling	52	60.9	55.9	56.26666667
PF14	ProFluorid	After pH cycling	45.4	46.6	41.6	44.53333333

PF15	ProFluorid	After pH cycling	44.4	45.7	45.3	45.13333333
PF16	ProFluorid	After pH cycling	41.4	42.8	47.8	44
PF17	ProFluorid	After pH cycling	52	52.9	56.9	53.93333333
PF18	ProFluorid	After pH cycling	37.3	48.2	43	42.83333333
PF19	ProFluorid	After pH cycling	68.8	69.9	54.9	64.53333333
PF20	ProFluorid	After pH cycling	47.9	56.9	46.7	50.5
PF1	ProFluorid	After Brushing	214.3	266.6	291	257.3
PF2	ProFluorid	After Brushing	313.4	342.6	342.6	332.8666667
PF3	ProFluorid	After Brushing	301	308	336.5	315.1666667
PF4	ProFluorid	After Brushing	258.2	311.6	306.2	292
PF5	ProFluorid	After Brushing	304.5	315.2	313.4	311.0333333
PF6	ProFluorid	After Brushing				
PF7	ProFluorid	After Brushing	338.5	340.6	309.8	329.6333333
PF8	ProFluorid	After Brushing	296	206.3	317.1	273.1333333
PF9	ProFluorid	After Brushing	326.6	311.6	336.5	324.9
PF10	ProFluorid	After Brushing	304.5	306.2	292.6	301.1
PF11	ProFluorid	After Brushing	286.2	221.7	248.9	252.2666667
PF12	ProFluorid	After Brushing	246.6	246.4	236.5	243.1666667
PF13	ProFluorid	After Brushing	241.4	220.6	201.5	221.1666667
PF14	ProFluorid	After Brushing	291	273.9	313.4	292.7666667
PF15	ProFluorid	After Brushing	279.9	210.2	200.6	230.2333333
PF16	ProFluorid	After Brushing	266.6	297.6	251.5	271.9
PF17	ProFluorid	After Brushing	201.5	289.4	237.7	242.8666667
PF18	ProFluorid	After Brushing	357.7	180.7	276.9	271.7666667
PF19	ProFluorid	After Brushing	227.2	238.9	210.2	225.4333333
PF20	ProFluorid	After Brushing	236.5	269.5	309.8	271.9333333
D1	Duraphat	Baseline	323.3	315.3	347.5	328.7
D2	Duraphat	Baseline	314	250.6	319.3	294.6333333
D3	Duraphat	Baseline	327.4	338.7	321.9	329.3333333
D4	Duraphat	Baseline	361.5	361.5	289.7	337.5666667
D5	Duraphat	Baseline	297.9	319.3	294.3	303.8333333
D6	Duraphat	Baseline	341.6	374.6	366.3	360.8333333
D7	Duraphat	Baseline	372.9	355.2	401.2	376.4333333
D8	Duraphat	Baseline	410.7	404.9	408.7	408.1
D9	Duraphat	Baseline	334.4	338.7	381.4	351.5
D10	Duraphat	Baseline	335.8	319.3	299.1	318.0666667
D11	Duraphat	Baseline	225.9	293.2	287.4	268.8333333
D12	Duraphat	Baseline	331.5	337.2	312.8	327.1666667

D13	Duraphat	Baseline	252.5	271.2	284.1	269.2666667
D14	Duraphat	Baseline	228.3	229.9	239.1	232.4333333
D15	Duraphat	Baseline	277.5	247.9	243.4	256.2666667
D16	Duraphat	Baseline	248.8	281.9	270.1	266.9333333
D17	Duraphat	Baseline	315.3	318	308.9	314.0666667
D18	Duraphat	Baseline	250.6	256.3	256.3	254.4
D19	Duraphat	Baseline	258.2	278.6	286.3	274.3666667
D20	Duraphat	Baseline	295.5	295.5	308.9	299.9666667
D1	Duraphat	After pH cycling	101.2	90.4	88	93.2
D2	Duraphat	After pH cycling	83.2	121.9	131.2	112.1
D3	Duraphat	After pH cycling	75.7	85	81.2	80.63333333
D4	Duraphat	After pH cycling	118.3	101.7	104.1	108.0333333
D5	Duraphat	After pH cycling	91.2	94.9	92	92.7
D6	Duraphat	After pH cycling	93.6	81.8	88.4	87.93333333
D7	Duraphat	After pH cycling	88.8	90	102.2	93.66666667
D8	Duraphat	After pH cycling	109.2	103.2	97.6	103.3333333
D9	Duraphat	After pH cycling	92	90.4	104.1	95.5
D10	Duraphat	After pH cycling	101.7	75.1	95.8	90.86666667
D11	Duraphat	After pH cycling	97.1	120.7	108.7	108.8333333
D12	Duraphat	After pH cycling	109.2	99.8	109.8	106.2666667
D13	Duraphat	After pH cycling	85.4	79.8	93.6	86.26666667
D14	Duraphat	After pH cycling	93.6	80.5	98.9	91
D15	Duraphat	After pH cycling	87.3	88.8	94.1	90.06666667
D16	Duraphat	After pH cycling	100.8	94.9	97.1	97.6
D17	Duraphat	After pH cycling	133.3	113.1	117.1	121.1666667
D18	Duraphat	After pH cycling	92.4	91.2	93.2	92.26666667
D19	Duraphat	After pH cycling	106.1	83.9	77.2	89.06666667
D20	Duraphat	After pH cycling	106.6	102.7	103.6	104.3
D1	Duraphat	After Brushing	273.9	381	317.1	324
D2	Duraphat	After Brushing	340.6	344.7	334.5	339.9333333
D3	Duraphat	After Brushing	315.2	319	309.8	314.6666667
D4	Duraphat	After Brushing	236.5	308	256.8	267.1
D5	Duraphat	After Brushing	241.4	315.2	304.5	287.0333333
D6	Duraphat	After Brushing	213.3	266.6	275.4	251.7666667
D7	Duraphat	After Brushing	388.4	346.8	322.7	352.6333333
D8	Duraphat	After Brushing	306.2	319	324.6	316.6

D9	Duraphat	After Brushing	291	260.9	276.9	276.2666667
D10	Duraphat	After Brushing	276.9	248.9	228.4	251.4
D11	Duraphat	After Brushing	272.4	281.5	260.9	271.6
D12	Duraphat	After Brushing	304.5	320.8	291	305.4333333
D13	Duraphat	After Brushing	243.8	263.7	279.9	262.4666667
D14	Duraphat	After Brushing	308	292.6	270.9	290.5
D15	Duraphat	After Brushing	235.3	238.9	313.4	262.5333333
D16	Duraphat	After Brushing	196.9	286.2	235.3	239.4666667
D17	Duraphat	After Brushing				
D18	Duraphat	After Brushing	242.6	302.8	292.6	279.3333333
D19	Duraphat	After Brushing	260.9	281.5	265.1	269.1666667
D20	Duraphat	After Brushing	226.1	349	319	298.0333333
PR1	Prevident	Baseline	335.8	383.1	384.9	367.9333333
PR2	Prevident	Baseline	399.3	414.6	408.7	407.5333333
PR3	Prevident	Baseline	430.7	416.5	424.5	423.9
PR4	Prevident	Baseline	390.2	414.6	434.9	413.2333333
PR5	Prevident	Baseline	372.9	384.9	406.8	388.2
PR6	Prevident	Baseline	367.9	359.9	369.6	365.8
PR7	Prevident	Baseline	379.7	392	374.6	382.1
PR8	Prevident	Baseline	408.7	406.8	418.5	411.3333333
PR9	Prevident	Baseline	410.7	408.7	418.5	412.6333333
PR10	Prevident	Baseline	410.7	412.6	412.6	411.9666667
PR11	Prevident	Baseline	397.5	406.8	395.6	399.9666667
PR12	Prevident	Baseline	386.6	392	401.2	393.2666667
PR13	Prevident	Baseline	301	294.3	323.3	306.2
PR14	Prevident	Baseline	393.8	406.8	401.2	400.6
PR15	Prevident	Baseline	256.3	277.5	285.2	273
PR16	Prevident	Baseline	295.5	265.1	281	280.5333333
PR17	Prevident	Baseline	386.6	366.3	364.7	372.5333333
PR18	Prevident	Baseline	422.5	414.6	420.5	419.2
PR19	Prevident	Baseline	299.1	305.2	290.9	298.4
PR20	Prevident	Baseline	422.5	408.7	439.1	423.4333333
PR1	Prevident	After pH cycling	40.8	51.2	138.4	76.8
PR2	Prevident	After pH cycling	92	62.7	52.9	69.2
PR3	Prevident	After pH cycling	71.6	71.3	67.5	70.13333333
PR4	Prevident	After pH cycling	88.4	69.4	69.6	75.8
PR5	Prevident	After pH cycling	76	71.9	74.2	74.03333333
PR6	Prevident	After pH cycling	81.8	68.8	71.3	73.96666667

PR7	Prevident	After pH cycling	66	67.8	77.6	70.46666667
PR8	Prevident	After pH cycling	98.5	102.7	105.6	102.2666667
PR9	Prevident	After pH cycling	80.8	76.6	90.4	82.6
PR10	Prevident	After pH cycling	90.8	70.5	73.3	78.2
PR11	Prevident	After pH cycling	62.9	85.8	90	79.56666667
PR12	Prevident	After pH cycling	79.2	92	88.8	86.66666667
PR13	Prevident	After pH cycling	59.8	79.5	70.5	69.93333333
PR14	Prevident	After pH cycling	78.2	71.6	99.8	83.2
PR15	Prevident	After pH cycling	52.7	71	67	63.56666667
PR16	Prevident	After pH cycling	78.5	68.6	64.8	70.63333333
PR17	Prevident	After pH cycling	99.4	83.2	84.3	88.96666667
PR18	Prevident	After pH cycling	65	60.2	68.1	64.43333333
PR19	Prevident	After pH cycling	100.3	96.2	94.1	96.86666667
PR20	Prevident	After pH cycling	81.2	62.2	62	68.46666667
PR1	Prevident	After Brushing	250.2	304.5	326.6	293.7666667
PR2	Prevident	After Brushing				
PR3	Prevident	After Brushing	412.1	404	371.4	395.8333333
PR4	Prevident	After Brushing	396.1	366.8	373.8	378.9
PR5	Prevident	After Brushing	364.5	328.5	353.3	348.7666667
PR6	Prevident	After Brushing	220.6	294.3	334.5	283.1333333
PR7	Prevident	After Brushing	255.5	315.2	230.6	267.1
PR8	Prevident	After Brushing	246.4	272.4	317.1	278.6333333
PR9	Prevident	After Brushing	304.5	369.1	311.6	328.4
PR10	Prevident	After Brushing	366.8	381	376.2	374.6666667
PR11	Prevident	After Brushing	256.8	272.4	320.8	283.3333333
PR12	Prevident	After Brushing	161.6	388.4	404	318
PR13	Prevident	After Brushing	306.2	299.3	342.6	316.0333333
PR14	Prevident	After Brushing	272.4	234.1	317.1	274.5333333
PR15	Prevident	After Brushing	234.1	238.9	226.1	233.0333333
PR16	Prevident	After Brushing	221.7	207.3	243.8	224.2666667
PR17	Prevident	After Brushing	278.4	294.3	304.5	292.4
PR18	Prevident	After Brushing	243.8	246.4	270.9	253.7
PR19	Prevident	After Brushing	281.5	256.8	289.4	275.9
PR20	Prevident	After Brushing	243.8	229.1	281.5	251.4666667
C1	Control	Baseline	289.7	308.9	295.5	298.0333333
C2	Control	Baseline	315.3	330.1	292	312.4666667
C3	Control	Baseline	285.2	294.3	297.9	292.4666667

C4	Control	Baseline	334.4	328.8	330.1	331.1
C5	Control	Baseline	288.6	355.2	361.5	335.1
C6	Control	Baseline	290.9	295.5	299.1	295.1666667
C7	Control	Baseline	323.3	327.4	327.7	326.1333333
C8	Control	Baseline	279.7	281.9	267.1	276.2333333
C9	Control	Baseline	283	278.6	293.2	284.9333333
C10	Control	Baseline	301.5	334.4	331.5	322.4666667
C11	Control	Baseline	308.9	292	286.3	295.7333333
C12	Control	Baseline	292	323.3	320.6	311.9666667
C13	Control	Baseline	272.2	290.2	293.2	285.2
C14	Control	Baseline	275.4	271.2	264.1	270.2333333
C15	Control	Baseline	229.1	226.7	225.1	226.9666667
C16	Control	Baseline	328.8	308.9	299.1	312.2666667
C17	Control	Baseline	318	306.4	304	309.4666667
C18	Control	Baseline	331.5	305.2	276.4	304.3666667
C19	Control	Baseline	294.3	328.8	320.6	314.5666667
C20	Control	Baseline	346	350.5	378	358.1666667
C1	Control	After pH cycling	8.3	19.8	23.9	17.33333333
C2	Control	After pH cycling	4.8	5.5	7.4	5.9
C3	Control	After pH cycling	8.6	4.8	6.9	6.766666667
C4	Control	After pH cycling	9.4	12.9	25.1	15.8
C5	Control	After pH cycling	11.6	10.5	11.3	11.13333333
C6	Control	After pH cycling	11.4	10.2	12.9	11.5
C7	Control	After pH cycling	11.1	13.4	21.2	15.23333333
C8	Control	After pH cycling	9.9	13.3	15.8	13
C9	Control	After pH cycling	19.2	10.9	15.1	15.06666667
C10	Control	After pH cycling	11.3	11.6	8.8	10.56666667
C11	Control	After pH cycling	11.7	10	11.2	10.96666667
C12	Control	After pH cycling	19	17.8	9	15.26666667
C13	Control	After pH cycling	10.8	11.5	14.1	12.13333333
C14	Control	After pH cycling	21.8	27.8	34.3	27.96666667
C15	Control	After pH cycling	9.7	10	10.3	10
C16	Control	After pH cycling	6.1	10.8	9.1	8.666666667
C17	Control	After pH cycling	6.2	4.5	9.3	6.666666667
C18	Control	After pH cycling	4.3	22.9	6.1	11.1
C19	Control	After pH cycling	8.1	10.1	12.9	10.36666667

C20	Control	After pH cycling	10.3	9.6	12.1	10.66666667
C1	Control	After Brushing	275.4	276.9	268	273.4333333
C2	Control	After Brushing				
C3	Control	After Brushing	242.6	265.1	212.3	240
C4	Control	After Brushing	326.6	270.9	287.8	295.1
C5	Control	After Brushing	238.9	199.7	233	223.8666667
C6	Control	After Brushing	304.5	255.5	324.6	294.8666667
C7	Control	After Brushing	357.7	322.7	245.1	308.5
C8	Control	After Brushing	240.1	289.4	250.2	259.9
C9	Control	After Brushing	206.3	254.2	200.6	220.3666667
C10	Control	After Brushing	262.3	292.6	242.6	265.8333333
C11	Control	After Brushing	289.4	287.8	265.1	280.7666667
C12	Control	After Brushing	315.2	248.9	292.6	285.5666667
C13	Control	After Brushing	291	322.7	278.4	297.3666667
C14	Control	After Brushing	193.3	153.2	227.2	191.2333333
C15	Control	After Brushing	238.9	278.4	320.8	279.3666667
C16	Control	After Brushing	328.5	297.6	299.3	308.4666667
C17	Control	After Brushing	262.3	286.2	291	279.8333333
C18	Control	After Brushing	225	265.1	246.4	245.5
C19	Control	After Brushing	309.8	273.9	315.2	299.6333333
C20	Control	After Brushing	229.5	278.4	322.7	276.8666667

Appendix B. Raw data for enamel-wear measurement

Sample	Varnish	Time	Wear in µm
F1	Fluor Protector S	1 month brushing	26.6666667
F2	Fluor Protector S	1 month brushing	28.6666667
F3	Fluor Protector S	1 month brushing	25.3333333
F4	Fluor Protector S	1 month brushing	28.6666667
F5	Fluor Protector S	1 month brushing	29.3333333
F6	Fluor Protector S	1 month brushing	30
F7	Fluor Protector S	1 month brushing	39
F8	Fluor Protector S	1 month brushing	32.3333333
F9	Fluor Protector S	1 month brushing	21.6666667
F10	Fluor Protector S	1 month brushing	30.6666667
F11	Fluor Protector S	1 month brushing	23.6666667
F12	Fluor Protector S	1 month brushing	
F13	Fluor Protector S	1 month brushing	23
F14	Fluor Protector S	1 month brushing	29.3333333
F15	Fluor Protector S	1 month brushing	26.3333333
F16	Fluor Protector S	1 month brushing	15.3333333
F17	Fluor Protector S	1 month brushing	25.6666667
F18	Fluor Protector S	1 month brushing	20.6666667
F19	Fluor Protector S	1 month brushing	29.3333333
F20	Fluor Protector S	1 month brushing	30.3333333
F1	Fluor Protector S	3 months brushing	31
F2	Fluor Protector S	3 months brushing	38.3333333
F3	Fluor Protector S	3 months brushing	29.3333333
F4	Fluor Protector S	3 months brushing	32
F5	Fluor Protector S	3 months brushing	34.3333333
F6	Fluor Protector S	3 months brushing	34
F7	Fluor Protector S	3 months brushing	42
F8	Fluor Protector S	3 months brushing	39.3333333
F9	Fluor Protector S	3 months brushing	31.6666667
F10	Fluor Protector S	3 months brushing	37
F11	Fluor Protector S	3 months brushing	30.3333333
F12	Fluor Protector S	3 months brushing	
F13	Fluor Protector S	3 months brushing	31.6666667
F14	Fluor Protector S	3 months brushing	40
F15	Fluor Protector S	3 months brushing	32.3333333
F16	Fluor Protector S	3 months brushing	22.6666667

F17	Fluor Protector S	3 months brushing	33.3333333
F18	Fluor Protector S	3 months brushing	23
F19	Fluor Protector S	3 months brushing	36
F20	Fluor Protector S	3 months brushing	36.6666667
V1	Vanish	1 month brushing	
V2	Vanish	1 month brushing	28.3333333
V3	Vanish	1 month brushing	30.3333333
V4	Vanish	1 month brushing	33
V5	Vanish	1 month brushing	41.6666667
V6	Vanish	1 month brushing	41
V7	Vanish	1 month brushing	31.3333333
V8	Vanish	1 month brushing	41
V9	Vanish	1 month brushing	26
V10	Vanish	1 month brushing	28.6666667
V11	Vanish	1 month brushing	60
V12	Vanish	1 month brushing	36.3333333
V13	Vanish	1 month brushing	52.6666667
V14	Vanish	1 month brushing	42.3333333
V15	Vanish	1 month brushing	38.6666667
V16	Vanish	1 month brushing	29.3333333
V17	Vanish	1 month brushing	40.3333333
V18	Vanish	1 month brushing	28.6666667
V19	Vanish	1 month brushing	27.3333333
V20	Vanish	1 month brushing	42.6666667
V1	Vanish	3 months brushing	
V2	Vanish	3 months brushing	30.6666667
V3	Vanish	3 months brushing	33.3333333
V4	Vanish	3 months brushing	40
V5	Vanish	3 months brushing	44.3333333
V6	Vanish	3 months brushing	50.3333333
V7	Vanish	3 months brushing	50.6666667
V8	Vanish	3 months brushing	49.3333333
V9	Vanish	3 months brushing	35
V10	Vanish	3 months brushing	42.3333333
V11	Vanish	3 months brushing	70.3333333
V12	Vanish	3 months brushing	50
V13	Vanish	3 months brushing	57.3333333
V14	Vanish	3 months brushing	58
V15	Vanish	3 months brushing	48.6666667

V16	Vanish	3 months brushing	31
V17	Vanish	3 months brushing	43
V18	Vanish	3 months brushing	31.3333333
V19	Vanish	3 months brushing	40
V20	Vanish	3 months brushing	44.3333333
N1	NUPRO White	1 month brushing	26
N2	NUPRO White	1 month brushing	30.6666667
N3	NUPRO White	1 month brushing	38
N4	NUPRO White	1 month brushing	
N5	NUPRO White	1 month brushing	30.6666667
N6	NUPRO White	1 month brushing	27.3333333
N7	NUPRO White	1 month brushing	32
N8	NUPRO White	1 month brushing	30.6666667
N9	NUPRO White	1 month brushing	32.3333333
N10	NUPRO White	1 month brushing	62.6666667
N11	NUPRO White	1 month brushing	29
N12	NUPRO White	1 month brushing	33.6666667
N13	NUPRO White	1 month brushing	30.6666667
N14	NUPRO White	1 month brushing	50.3333333
N15	NUPRO White	1 month brushing	33.3333333
N16	NUPRO White	1 month brushing	33.6666667
N17	NUPRO White	1 month brushing	23.3333333
N18	NUPRO White	1 month brushing	29.3333333
N19	NUPRO White	1 month brushing	22
N20	NUPRO White	1 month brushing	29
N1	NUPRO White	3 months brushing	36.3333333
N2	NUPRO White	3 months brushing	32.3333333
N3	NUPRO White	3 months brushing	44
N4	NUPRO White	3 months brushing	
N5	NUPRO White	3 months brushing	35.3333333
N6	NUPRO White	3 months brushing	32.6666667
N7	NUPRO White	3 months brushing	35.3333333
N8	NUPRO White	3 months brushing	34.6666667
N9	NUPRO White	3 months brushing	46
N10	NUPRO White	3 months brushing	77
N11	NUPRO White	3 months brushing	36.6666667
N12	NUPRO White	3 months brushing	43.6666667
N13	NUPRO White	3 months brushing	40.6666667
N14	NUPRO White	3 months brushing	59.3333333

N15	NUPRO White	3 months brushing	39.6666667
N16	NUPRO White	3 months brushing	40.6666667
N17	NUPRO White	3 months brushing	28
N18	NUPRO White	3 months brushing	34
N19	NUPRO White	3 months brushing	31.3333333
N20	NUPRO White	3 months brushing	32.6666667
PF1	ProFluorid	1 month brushing	43
PF2	ProFluorid	1 month brushing	44.3333333
PF3	ProFluorid	1 month brushing	42.6666667
PF4	ProFluorid	1 month brushing	41.3333333
PF5	ProFluorid	1 month brushing	85.3333333
PF6	ProFluorid	1 month brushing	
PF7	ProFluorid	1 month brushing	34.6666667
PF8	ProFluorid	1 month brushing	52
PF9	ProFluorid	1 month brushing	38.3333333
PF10	ProFluorid	1 month brushing	40.6666667
PF11	ProFluorid	1 month brushing	47
PF12	ProFluorid	1 month brushing	36.6666667
PF13	ProFluorid	1 month brushing	32
PF14	ProFluorid	1 month brushing	36.6666667
PF15	ProFluorid	1 month brushing	32.3333333
PF16	ProFluorid	1 month brushing	31
PF17	ProFluorid	1 month brushing	40.3333333
PF18	ProFluorid	1 month brushing	65.6666667
PF19	ProFluorid	1 month brushing	62.6666667
PF20	ProFluorid	1 month brushing	37.6666667
PF1	ProFluorid	3 months brushing	49.6666667
PF2	ProFluorid	3 months brushing	51
PF3	ProFluorid	3 months brushing	47
PF4	ProFluorid	3 months brushing	57.6666667
PF5	ProFluorid	3 months brushing	94.3333333
PF6	ProFluorid	3 months brushing	
PF7	ProFluorid	3 months brushing	39
PF8	ProFluorid	3 months brushing	56.3333333
PF9	ProFluorid	3 months brushing	47.6666667
PF10	ProFluorid	3 months brushing	44
PF11	ProFluorid	3 months brushing	51
PF12	ProFluorid	3 months brushing	44.6666667
PF13	ProFluorid	3 months brushing	53.6666667

PF14	ProFluorid	3 months brushing	42
PF15	ProFluorid	3 months brushing	40.6666667
PF16	ProFluorid	3 months brushing	40
PF17	ProFluorid	3 months brushing	44.3333333
PF18	ProFluorid	3 months brushing	73.6666667
PF19	ProFluorid	3 months brushing	67
PF20	ProFluorid	3 months brushing	40.3333333
D1	Duraphat	1 month brushing	28.3333333
D2	Duraphat	1 month brushing	25.3333333
D3	Duraphat	1 month brushing	24.3333333
D4	Duraphat	1 month brushing	21.3333333
D5	Duraphat	1 month brushing	23
D6	Duraphat	1 month brushing	24.3333333
D7	Duraphat	1 month brushing	32
D8	Duraphat	1 month brushing	20.6666667
D9	Duraphat	1 month brushing	31
D10	Duraphat	1 month brushing	39.3333333
D11	Duraphat	1 month brushing	20.3333333
D12	Duraphat	1 month brushing	24.3333333
D13	Duraphat	1 month brushing	30.3333333
D14	Duraphat	1 month brushing	31.6666667
D15	Duraphat	1 month brushing	37.3333333
D16	Duraphat	1 month brushing	21.3333333
D17	Duraphat	1 month brushing	
D18	Duraphat	1 month brushing	27.3333333
D19	Duraphat	1 month brushing	21.6666667
D20	Duraphat	1 month brushing	16
D1	Duraphat	3 months brushing	34
D2	Duraphat	3 months brushing	31
D3	Duraphat	3 months brushing	35
D4	Duraphat	3 months brushing	24.6666667
D5	Duraphat	3 months brushing	28.3333333
D6	Duraphat	3 months brushing	32
D7	Duraphat	3 months brushing	38.6666667
D8	Duraphat	3 months brushing	33
D9	Duraphat	3 months brushing	33
D10	Duraphat	3 months brushing	43.6666667
D11	Duraphat	3 months brushing	26.3333333
D12	Duraphat	3 months brushing	30

D13	Duraphat	3 months brushing	35
D14	Duraphat	3 months brushing	33.6666667
D15	Duraphat	3 months brushing	47.6666667
D16	Duraphat	3 months brushing	29.6666667
D17	Duraphat	3 months brushing	
D18	Duraphat	3 months brushing	35
D19	Duraphat	3 months brushing	32.6666667
D20	Duraphat	3 months brushing	29
PR1	Prevident	1 month brushing	40
PR2	Prevident	1 month brushing	
PR3	Prevident	1 month brushing	33.3333333
PR4	Prevident	1 month brushing	52.6666667
PR5	Prevident	1 month brushing	31
PR6	Prevident	1 month brushing	53.6666667
PR7	Prevident	1 month brushing	58
PR8	Prevident	1 month brushing	41.3333333
PR9	Prevident	1 month brushing	31.3333333
PR10	Prevident	1 month brushing	33.3333333
PR11	Prevident	1 month brushing	41.6666667
PR12	Prevident	1 month brushing	26.6666667
PR13	Prevident	1 month brushing	30.6666667
PR14	Prevident	1 month brushing	31.6666667
PR15	Prevident	1 month brushing	29.3333333
PR16	Prevident	1 month brushing	37.3333333
PR17	Prevident	1 month brushing	45.6666667
PR18	Prevident	1 month brushing	28.6666667
PR19	Prevident	1 month brushing	27.3333333
PR20	Prevident	1 month brushing	30.3333333
PR1	Prevident	3 months brushing	50
PR2	Prevident	3 months brushing	
PR3	Prevident	3 months brushing	39.6666667
PR4	Prevident	3 months brushing	55
PR5	Prevident	3 months brushing	41.3333333
PR6	Prevident	3 months brushing	58.6666667
PR7	Prevident	3 months brushing	64.6666667
PR8	Prevident	3 months brushing	45.3333333
PR9	Prevident	3 months brushing	36
PR10	Prevident	3 months brushing	42.3333333
PR11	Prevident	3 months brushing	55

PR12	Prevident	3 months brushing	34.6666667
PR13	Prevident	3 months brushing	36.6666667
PR14	Prevident	3 months brushing	42
PR15	Prevident	3 months brushing	38
PR16	Prevident	3 months brushing	39.3333333
PR17	Prevident	3 months brushing	55
PR18	Prevident	3 months brushing	47
PR19	Prevident	3 months brushing	33.6666667
PR20	Prevident	3 months brushing	40.6666667
C1	Control	1 month brushing	34.6666667
C2	Control	1 month brushing	
C3	Control	1 month brushing	48.3333333
C4	Control	1 month brushing	46.6666667
C5	Control	1 month brushing	73.3333333
C6	Control	1 month brushing	57.6666667
C7	Control	1 month brushing	44.3333333
C8	Control	1 month brushing	57.6666667
C9	Control	1 month brushing	40
C10	Control	1 month brushing	45.6666667
C11	Control	1 month brushing	57
C12	Control	1 month brushing	48.3333333
C13	Control	1 month brushing	66.6666667
C14	Control	1 month brushing	39
C15	Control	1 month brushing	41.6666667
C16	Control	1 month brushing	43
C17	Control	1 month brushing	49.6666667
C18	Control	1 month brushing	49
C19	Control	1 month brushing	71.3333333
C20	Control	1 month brushing	60.3333333
C1	Control	3 months brushing	68
C2	Control	3 months brushing	
C3	Control	3 months brushing	73
C4	Control	3 months brushing	69.6666667
C5	Control	3 months brushing	80.3333333
C6	Control	3 months brushing	70.3333333
C7	Control	3 months brushing	54
C8	Control	3 months brushing	70
C9	Control	3 months brushing	41.6666667
C10	Control	3 months brushing	52.3333333

C11	Control	3 months brushing	61.3333333
C12	Control	3 months brushing	79.6666667
C13	Control	3 months brushing	73.3333333
C14	Control	3 months brushing	42.6666667
C15	Control	3 months brushing	56.6666667
C16	Control	3 months brushing	53
C17	Control	3 months brushing	59
C18	Control	3 months brushing	53.6666667
C19	Control	3 months brushing	90.6666667
C20	Control	3 months brushing	80.3333333